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Determination of Nitroaromatic Compounds and Isophorone in Industrial and Municipal Wastewaters

Battelle Columbus Labs., OH

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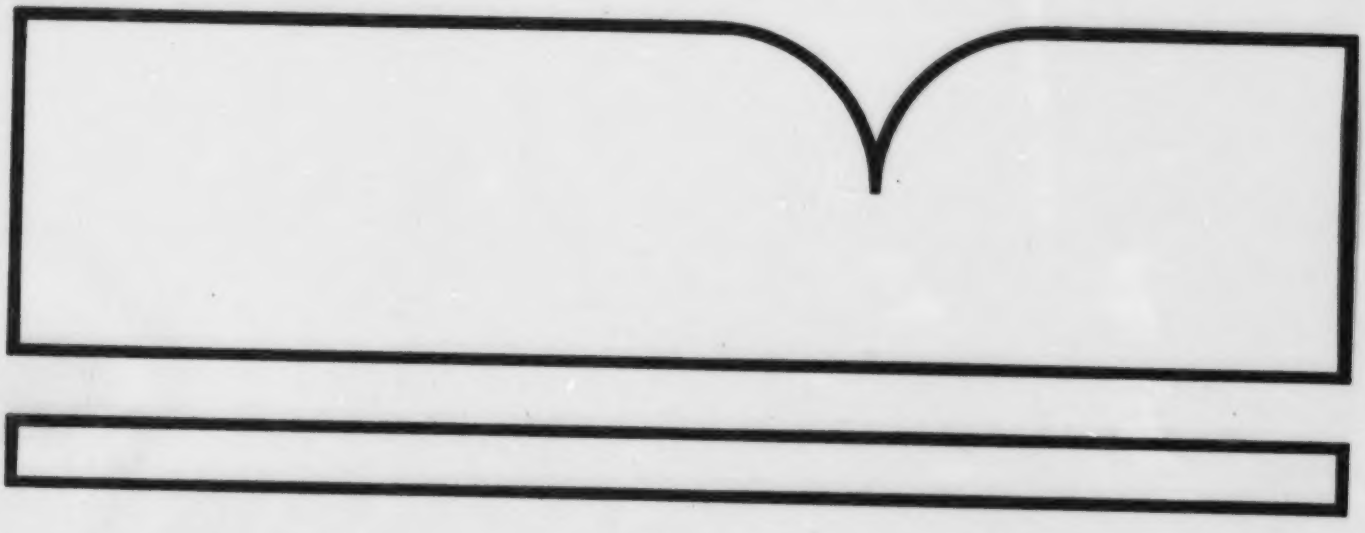

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DETERMINATION OF NITROAROMATIC COMPOUNDS AND ISOPHORONE IN INDUSTRIAL AND MUNICIPAL WASTEWATERS

by

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16. ABSTRACT

A method was developed for the determination of nitrobenzene, 2,4- and 2,6-dinitrotoluene (DNT), and isophorone in wastewaters. The methods development program consisted of: a literature review, determination of the stability of the compounds in organic solutions, determination of extraction efficiency for each compound from water using two organic solvents, determination of storage stability of each compound in water, and evaluation of various clean up techniques.

The final method was applied to several representative wastewaters spiked with the compounds at appropriate levels, as well as to surface water, in order to determine precision and accuracy of the method. For a wastewater sample spiked with 96 ppb of nitrobenzene, 560 ppb of isophorone and 8 ppb of 2,4-DNT, and 7 ppb of 2,6-DNT, recoveries were 70 ± 6% for nitrobenzene, 71 ± 5% for isophorone, 70 ± 2% for 2,4-DNT, and 78 ± 1% for 2,6-DNT. Minimum detectable levels in this wastewater are estimated to be 5 ppb for nitrobenzene, 5 ppb for isophorone, and 0.05 ppb for 2,4- and 2,6-DNT. However, caution must be used in presuming these MDLs are accurate for other wastewaters since several wastewaters were encountered which exhibited high levels of interfering compounds. For some complex wastewaters it may be necessary to apply additional clean up procedures or more selective analytical detection systems in order to achieve these levels of sensitivity.

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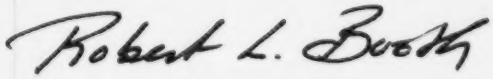
FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water. Conduct studies to determine the responses of aquatic organisms to water quality.
- Conduct an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

Under provisions of the Clean Water Act, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 priority pollutants and classes of pollutants to be toxic under Section 307(a) of the Act. This report is one of a series that investigates the analytical behavior of selected priority pollutants and suggests a suitable test procedure for their measurement.



Robert L. Booth, Acting Director
Environmental Monitoring and Support
Laboratory - Cincinnati

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ABSTRACT

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This report was submitted in fulfillment of Contract No. 68-03-2624 by Battelle Columbus Laboratories under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from November 1, 1977, to March 1, 1979, and work was completed as of March 1, 1979.

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SECTION 1

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has established a list of 129 toxic substances which are either known or likely to be present in selected industrial aqueous effluents. The 114 organic substances have been categorized on a chemical basis into 12 categories. The EPA has established a monitoring program for the purpose of controlling the concentration of such substances in the effluents at an environmentally acceptable level. In support of this program, analytical methodologies for these toxic substances have been developed and evaluated by application to some selected effluents.

This is a report of the development and evaluation of methodology for the determination of isophorone, nitrobenzene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene which represent the Category 4 compounds in industrial aqueous discharges. The major thrust of the work is in gas chromatography (GC) using flame ionization (FID) and electron capture detection, (ECD) but high performance liquid chromatography (HPLC) has been evaluated as a possible method using both UV and ECD.

The successful completion of this program involved the fulfillment of certain directives set forth in the contract by EPA. An extensive literature review was first conducted to evaluate the previous work in the area. Subsequent work was directed toward determination and then full evaluation of an appropriate measurement technique, which best satisfied the requirements for sensitivity and selectivity, as well as the considerations of sample cost, that is, equipment, time, and training which would be needed for the method. The stability of the nitroaromatic compounds and isophorone in water miscible solvents and their stability in chlorinated and unchlorinated buffered water at different pHs and storage temperatures were studied over the prescribed time periods. Extraction efficiency of two organic solvents was also studied for the standard compounds. The remainder of the program involved the study of the sample preparation and clean up steps which would be necessary to eliminate sample interferences. The complete method was then applied to several representative wastewater samples and an assessment was made of the precision and accuracy of the complete procedure.

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SECTION 2

LITERATURE REVIEW

An automated computer search was made of Chemical Abstracts, Biological Abstracts, Analytical Abstracts, and National Technical Information Services. Only a few articles were found pertaining to the analysis of nitroaromatic compounds in water. Two of these articles described analytical methods based on the direct colorimetric determination of nitrate[1] or a Meisenheimer complex[2] formed from the parent nitroaromatic compound. Since the detection limits of these methods are in the 10-20 ppm region they are not useful for wastewater analysis where ppb detection limits are sought. A third article described a method for determining TNT in sea water[3]. This method involves extraction with benzene followed by analysis with an electron capture gas chromatograph using a Dexsil 300 stationary phase. Approximately 70% extraction efficiency was obtained at the 1 ppb level and the detection limit was reported to be 20 ppt.

Several other articles were located which dealt with the gas chromatographic determination of nitroaromatics in media other than water. Several of these used flame ionization detection[4,5,6] and lacked the sensitivity for most wastewater applications. One article[7] reported the use of thin layer chromatography for the determination of 1,3,5-trinitrobenzene, 1,2-dinitrobenzene, and several trinitrotoluene isomers as well as other nitro compounds. Absolute detection limits were in the picogram region. No articles were found dealing with the HPLC analysis of the Category 4 compounds in water nor were any articles found dealing with the analysis of isophorone in aqueous media.

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SECTION 3

EXPERIMENTAL PROCEDURES

EVALUATION OF MEASUREMENT TECHNIQUES

Gas Chromatography (GC)

Isothermal, linear, and step-temperature programs were run on 1.95% QF-1/1.5% OV-17 (Gas Chrom Q 80/100 mesh), 3.0% OV-17 (Chromosorb W 80/100 mesh), 3.0% OV-1 (Chromosorb W 80/100 mesh), and 3.0% OV-101 (Chromosorb W 80/100 mesh). Glass columns of 1/4" O.D. x 4' were used for all stationary phases with the exception of OV-101 which was packed into a 1/4" O.D. x 10' column. A Hewlett-Packard 5830A chromatograph was used with microprocessor controlled temperature programming and peak integration for quantitation.

The mobile phase for the FID was nitrogen and for the ECD, 90% Argon/methane, which was passed through a heated filter and oxygen scrubber. The FID was kept at 250°C, the ECD (^{63}Ni source) was set at 330°C, and the injector temperature remained at 250°C. Manual injections were made with a 10 µL Hamilton syringe.

All standard solutions were prepared with Burdick and Jackson solvents. The analytical grade standards were purchased from Aldrich Chemical Company. Nitrobenzene was used as received. Isophorone was distilled under vacuum with a water aspirator connected to the distillation apparatus. A colorless fraction (b.p. 213°-214°C at S.T.P.) was collected and stored at 4°C. The 2,6- and 2,4-dinitrotoluenes were recrystallized from benzene by addition of hexane. Melting points were 71°C for 2,4-dinitrotoluene and 66°C for 2,6-dinitrotoluene.

As discussed later in this report, the 1.95% QF-1/1.5% OV-17 was found to be the most suitable stationary phase for this analysis. Using this column the linearity and sensitivity for each compound was investigated using both FID and ECD and the chromatographic conditions described above. The column temperature was 85°C for isophorone and nitrobenzene and 145°C for 2,4-DNT and 2-6-DNT. The conditions were chosen since it appeared impractical to determine all four compounds in a single isothermal run due to the wide range of retention times. To determine the linear range for each compound, four microliter volumes of standard solutions of the compounds at various concentrations, in acetone, were injected. The quantity of material necessary to produce a peak with a signal to noise ratio of about 5:1 was defined as the minimum detectable limit (MDL). Solution concentrations were chosen to cover the range from the MDL to the

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upper limit of linearity. The results of this study are presented in a separate section of this report.

High Performance Liquid Chromatography (HPLC)

A preliminary investigation was conducted in order to determine whether or not HPLC would be an appropriate technique for the determination of these compounds. Previous work at Battelle[8] showed that reverse phase chromatography was not selective enough for the separation of 2,4- and 2,6-DNTs. Furthermore, the use of electron capture detection restricted studies to normal phase because of the electron affinity of reverse phase solvents. Thus, only normal phase chromatography was examined in this study.

The following HPLC conditions were used:

Solvent delivery system - Varian Model 8500
Stationary phase - Dupont silica gel, 4.6 mm I.D. x 250 mm long, 10 micron particle diameter
Mobile phase - 3% ethyl acetate in pentane
Flow rate - 2.3 mL/min
Injection volume - 10 µL
Detection system - UV - Dupont Model 837. ECD - Pye Unicam model LC-EC Detector unit.

A comparison was made of the sensitivity of the two detectors for the Category 4 compounds by injecting a series of standard solutions at appropriate concentrations in hexane.

SOLVENT STABILITY STUDIES

Solutions containing 200 ppm of all four compounds in acetone and methanol were prepared. The solutions were flame sealed in 10 mL glass ampules. Immediately after sealing and after storage in the dark and at room temperature for 30, 60, and 90 days, three ampules of each solution were opened and assayed by triplicate injection of four microliter volumes onto the GC/FID system.

Quantitative analysis of the samples was done by comparison of the peak areas which were obtained from the microprocessor on the HP5830 GC to those of a 200 ppm solution freshly prepared in the same solvent as the sample to be analyzed. The chromatographic conditions used are as follows:

GC - HP5830A
Column - 1.95% QF-1/1.5% OV-17 on Gas Chrom Q (80/100 mesh) 1/4" O.D. x 6'.
Flow rate - 44 mL/min, Helium
Injector temperature - 250°C
Detector temperature - 250°C
Column temperature - 80°-180°C at 8°C/min

Statistical evaluation of this data was made with the computer program, SPSS-Statistical Package for the Social Sciences, Northwestern University

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Version 6.5. The subprogram "oneway" provided fundamental statistics of the data besides oneway analysis of variance (ANOVA) for each compound due to changes in the concentration as a function of time.

EXTRACTION STUDIES

The solvents methylene chloride and ethyl acetate were examined for the extraction of Category 4 compounds from aqueous solutions. Extraction at pH 2,7, and 10 was made with methylene chloride and ethyl acetate in triplicate for all pHs. A 200 µL volume of a 100 ppb solution was added to a 500 mL buffered aqueous phase and extracted with three 100 mL quantities of organic solvent. After drying the solvent by filtration through anhydrous $MgSO_4$, the extract was evaporated to about 10 mL on a rotating evaporator and then further concentrated to about 200 µL on a vortex evaporator at 30°C. The dosage volume and 200 µL final volume was measured in a 1-mL syringe calibrated in 10 µL increments (Hamilton No. 1001). At no time during the procedure did the samples reach a temperature above 30°C. Buffers were purchased from VWR Scientific to maintain the three pH levels. Their compositions are as follows:

Buffer Systems

pH	Composition	Ionic Strength
2.0	Tartaric and Phosphoric Acids and Potassium Biphthalate	.05M
7.0	Sodium and Potassium Phosphate	.05M
10.0	Sodium Borate and Sodium Carbonate	.05M

Microcentral Laboratories, the manufacturer of the capsules, refused to furnish the absolute quantities of each component in the buffer. Concentration levels were detemined with GC-FID by comparison to an external standard using the chromatographic conditions described in the previous section.

Several other experiments were conducted to validate the use of alternate extraction and work up schemes. In order to determine if smaller volumes of solvent and/or alternate drying agents gave comparable results the following experiments was conducted.

A 500-mL volume of water (buffered at pH 7 as above) was spiked with 200 µL of a 100 ppm acetone solution of the four compounds. Extraction was done with three 30 mL volumes of methylene chloride followed by drying the extract by addition of two grams of Na_2SO_4. The extract was then decanted and concentrated to 10 mL on a rotary evaporator and then reduced in volume further to 200 µL by vortex evaporation. GC-FID was used for quantitation. This experiment was done in triplicate and repeated using

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$MgSO_4$ as the drying agent. The effect of solvent volume was determined by comparison of these recoveries to those in the previous study, where 100 µL volumes were used.

In order to determine if K-D concentration was as effective as vortex concentration, a 2 mL volume of an acetone solution containing 200 ppm of each compound was diluted to 10 mL with methylene chloride. The solution was then concentrated to 2 mL by vortex evaporation at 30°C or by K-D concentration using a Kontes tube heater at about 100°C. Each concentration procedure was done in triplicate and the final concentrate assayed by GC-FID as described earlier.

A third experiment was conducted to determine if any loss of these compounds resulted during solvent displacement of the methylene chloride with toluene, since this is necessary when GC-ECD is used. A 500 µL volume of a 200 ppm acetone solution of the compounds was diluted to 10 mL with methylene chloride. The solution was then concentrated to 1 mL by K-D using a tube heater at 100°C. Five-hundred microliters of toluene were then added and the sample further concentrated to 500 µ. This concentrate was then analyzed by GC-FID as before. This experiment was done in triplicate.

PRESERVATION STUDIES

In order to examine the stability of these compounds under various storage conditions the following experiment was conducted. Samples were prepared by dosing 500 mL buffered water with 200 µL of an acetone solution containing 100 ppm of each compound. All samples were stored in the dark. Two temperatures, room and 4°C, three pH levels, 2, 7, and 10, and two chlorine levels, 0 and 2 ppm were investigated. This resulted in a 3 x 2 x 2 storage condition matrix, in which each point in the matrix was run in duplicate, giving a total of 24 samples which were studied. The three pH levels were achieved using the buffers described in the Extraction Section. The two ppm chlorine level was achieved by spiking the sample with 2 mL of a solution containing 1500 ppm of calcium hypochlorite.

After storage for seven days, the samples were extracted using the same extraction and work up procedures as were used for the extraction studies described earlier, except that 30 mL quantities of methylene chloride were used instead of 100 mL quantities. Quantitation was done by GC-FID using an external standard as described previously. ANOVA analysis was done on the data using the SPSS computer package previously described.

CLEAN UP METHODS EVALUATION

Both Florisil and silica gel were evaluated for the elution of Category 4 compounds. Florisil (Sigma Chemical Company, 60-100/PF) and silica gel (Davidson Chemical Company, 100-200 mesh, Grade H) were prepared by wet packing 10 gram quantities of these stationary phases (baked at 120°C for 24 hours) as a slurry in $MeCl_2$. The columns were then eluted with 50 mL of pentane. A 500 µL volume of a 5% $MeCl_2$/pentane solution, containing 2 mg/mL of each compound was added to the top of the column. Both columns were then eluted with a series of solvents as shown in Table 1

(Conditions 1 and 2) and the fractions analyzed by GC-FID as described previously.

After examination of the separation profiles of each column, Florisil was selected as the most appropriate for this application. From the point of view of sample concentration acetone was considered to be preferable to MeOH, since it is more volatile. Therefore, the elution profile of the Category 4 compounds was determined using a series of acetone/$MeCl_2$ mixtures as shown in Table 1, Condition 3. This elution scheme was used for the analysis of the first three wastewater samples described in the next section.

METHOD VALIDATION STUDIES

Six water samples were received and used to validate the proposed test procedure for Category 4 compounds. These samples were as follows:

1. Sewer pump sample receiving wastes from phenolic resins, vinyl acetate, and polyvinyl chloride process areas.

2. Brine sample from holding tank receiving washings from ships delivering various commodities.

3. Secondary sewage effluent (Columbus, Ohio).

4. Final effluent from UNOX treatment system receiving wastes from plants producing plasticizers, butyl rubber, and olefins.

5. Surface water (Olentangy River).

6. Final effluent from organic chemical plants producing nitrobenzene, o-dichlorobenzene, o-nitrophenol, aniline, and oil additives.

For the validation of the protocol the experimental design was as follows. Three replicates were run undosed initially. Based on these results the sample was then dosed with levels of the compounds sufficient to give a response at least five times the background level and rerun in triplicate. After dosing at the same level, three aliquots of the sample were placed in the refrigerator at 4°C for seven days. The samples were then reassayed.

The methodology used in the analysis of samples 3-6 is presented in the Appendix and is the optimized protocol resulting from this work. The methodology used for samples 1 and 2 differed from the final protocol in two respects. The clean up method used for samples 1 and 2 was Condition 3 in Table 1, presented in the Clean up Methods evaluation section. This method was replaced with clean up Condition 4, Table 2, which was used for samples 3-6 and is included in the final method. This method involved activation of the Florisil at 200°C, rather than 120°C (as in Condition 3). The elution solvent was also changed, as shown in Table 2, to elute the compounds with 30 mL of 10% Acetone/$MeCl_2$. This procedure gave a more reliable elution profile than the previous condition.

TABLE 1. COLUMN CHROMATOGRAPHIC SCHEME

Condition	Stationary Phase	Fraction Number	Mobile Phase
			10 mL volumes
1	Silica Gel	1.	Pentane
		2.	5% $MeCl_2$/Pentane
		3.	20% $MeCl_2$/Pentane
		4.	50% $MeCl_2$/Pentane
		5.	100% $MeCl_2$
		6.	20% MeOH/$MeCl_2$
		7.	50% MeOH/$MeCl_2$
		8.	50% MeOH/$MeCl_2$
		9.	50% MeOH/$MeCl_2$
			10 mL volumes
2	Florisil	1.	Pentane
		2.	5% $MeCl_2$/Pentane
		3.	20% $MeCl_2$/Pentane
		4.	50% $MeCl_2$/Pentane
		5.	100% $MeCl_2$
		6.	20% MeOH/$MeCl_2$
		7.	50% MeOH/$MeCl_2$
		8.	50% MeOH/$MeCl_2$
		9.	50% MeOH/$MeCl_2$
			10 mL volumes
3	Florisil	1.	30% Acetone/$MeCl_2$
		2.	40% Acetone/$MeCl_2$
		3.	50% Acetone/$MeCl_2$
		4.	60% Acetone/$MeCl_2$
		5.	70% Acetone/$MeCl_2$
		6.	80% Acetone/$MeCl_2$

TABLE 2. MODIFIED CLEAN UP METHOD

Condition	Stationary Phase	Mobile Phase
4	Florisil Activated at 200°C	30 mL each 1. $MeCl_2$ 2. 10% Acetone/$MeCl_2$ 3. 10% Acetone/$MeCl_2$

For samples 1-3 nitrobenzene and isophorone were determined by isothermal elution at 85°C with ECD, whereas for samples 4-6 they were determined by FID, as described in the Appendix. A detailed description of the treatment and analytical consideration for each sample is given in the results section.

The basis for selection of these particular samples was as follows. Samples 1 and 2 were obtained from a source which had previously been found to contain isophorone. Sample 3 was chosen at the request of the EPA project officer, who asked that the wastewater application phase for all categories include a secondary municipal sewage effluent. Sample 5 was chosen in order to validate the method for surface waters as well. Sample 4 was designated by the EPA project officer since it represents an effluent from an advanced waste treatment system serving a wide variety of chemical processes. Sample 6 was a common effluent from several organic chemical manufacturing plants and was believed to contain nitrobenzene.

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SECTION 4

RESULTS AND DISCUSSION

EVALUATION OF MEASUREMENT TECHNIQUES

Gas Chromatography (GC)

The retention times of the nitroaromatic compounds and isophorone for the GC columns tested are shown in Table 3. The OV-1 column packing exhibits the least affinity for Category 4 compounds which is to be expected since it is the least polar of the packing materials studied. Separation of the four compounds was accomplished with 3.0% OV-1, 3% OV-101, and 1.95% QF-1/1.5% OV-17. However, 3% OV-17 did not separate isophorone and nitrobenzene. The chromatograms shown in Figures 1-3, demonstrate the type of separation that was achieved with these stationary phases. The 1.95% QF-1/1.5% OV-17 stationary phase was chosen for use in the methodology over the others because it showed less solvent peak tailing under the conditions used. For confirmation purposes, either OV-101 or OV-17 is suitable, however, it must be kept in mind that isophorone and nitrobenzene are not resolved on OV-17. OV-17 gives better peak slopes with less tailing for 2,4-DNT and 2,6-DNT than does OV-1.

The response curves for the four compounds using FID and ECD (using 1.95% QF-1/1.5% OV-17) are shown in Figures 4-6. The limits of detection for these compounds on both detectors are shown in Table 4. From these data it is readily apparent that ECD is the preferred detector for 2,4-DNT and 2,6-DNT due to its extremely high sensitivity for these compounds. The sensitivity of the two detectors for isophorone is comparable. Initially, it was felt that ECD would be more selective for isophorone and thus it was chosen as the preferred detector. However, it was later determined for certain wastewater samples that FID was more appropriate for isophorone determination since it exhibited a lower background level. The ECD is more sensitive for nitrobenzene than FID and is thus the preferred detector. However, the use of FID for nitrobenzene has the advantage that, since both isophorone and nitrobenzene can be determined at the same isothermal elution temperature, only one chromatographic run needs to be made to determine both compounds.

The linearity of the chromatographic column was established down to 9.0 nanograms injected for nitrobenzene, 0.12 for 2,6-DNT, and 0.12 for 2,4-DNT using the ECD, and 4 ng injected for isophorone and nitrobenzene using FID.

Due to the wide difference in retention times for the four compounds, it

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TABLE 3. RETENTION TIMES OF CATEGORY 4 COMPOUNDS ON VARIOUS STATIONARY PHASES

Column	Temperature (°C)	Flow (mL/min)	Retention Times (min)	Compound
1.95% QF-1/ 1.5% OV-17	85	44	3.31	Nitrobenzene
			4.49	Isophorone
	145	44	3.52	2,6-DNT
			5.35	2,4-DNT
3% OV-101*	100	44	4.31	Nitrobenzene
			5.72	Isophorone
	150	44	4.75	2,6-DNT
			6.54	2,4-DNT
3% OV-1	100	30	1.27	Nitrobenzene
			1.61	Isophorone
			8.14	2,6-DNT
			12.4	2,4-DNT
OV-17	120	30	5.50	Nitrobenzene
	150		5.50	Isophorone
			16.7	2,4-DNT
			22.8	2,6-DNT

*1/4" x 10' glass column, all others were 1/4" x 4' glass column.

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TABLE 4. DETECTION LIMITS FOR CATEGORY 4 COMPOUNDS

Compound	Detector	Minimum detectable limit* (ng injected)
Nitrobenzene	FID	2.5
Isophorone		3.0
2,6-DNT		2.9
2,4-DNT		3.0
Nitrobenzene	ECD	1.2
Isophorone		1.1
2,6-DNT		0.06
2,4-DNT		0.04

*Calculated using five to one signal to noise ratio.

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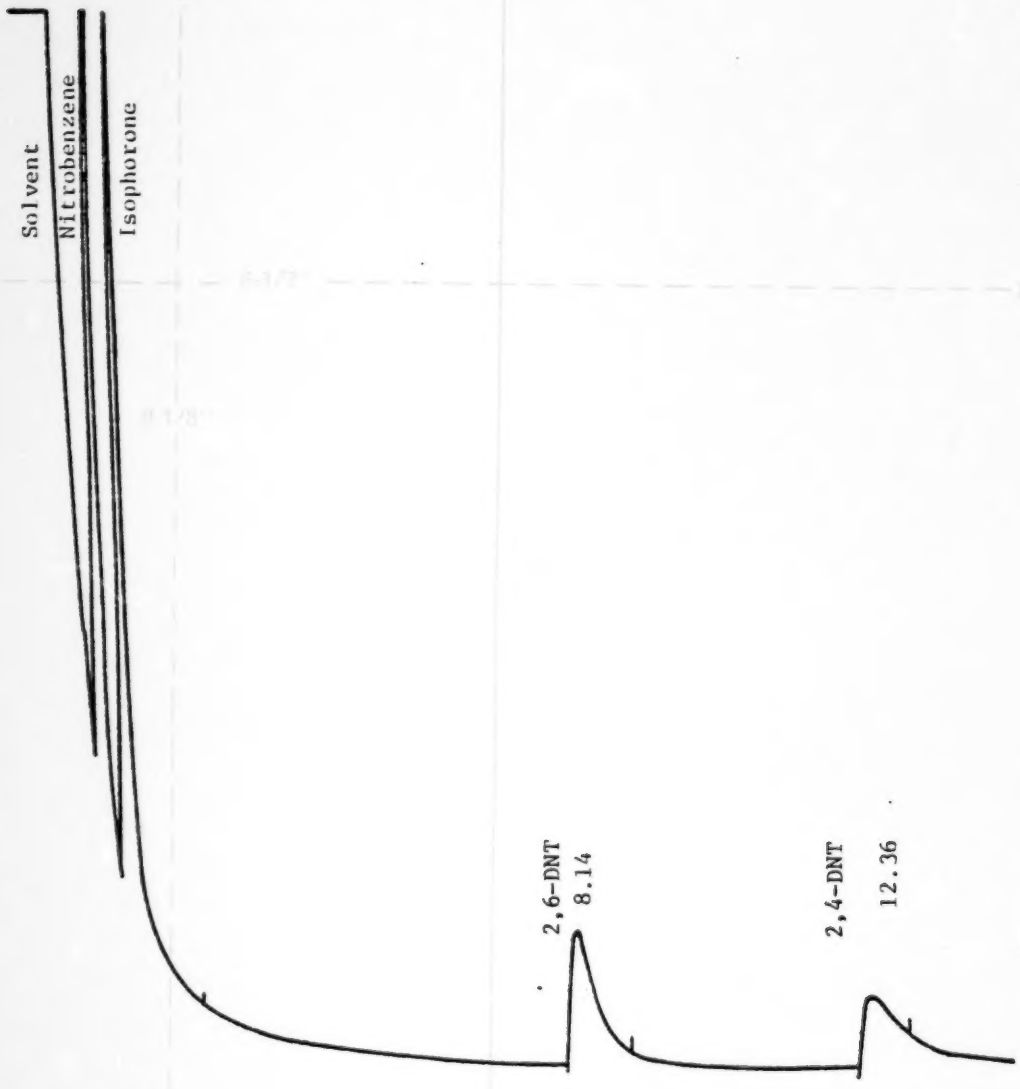

Figure 1. Chromatographic separation of the category 4 compounds at 100° on OV-1 at the 400 ng level.

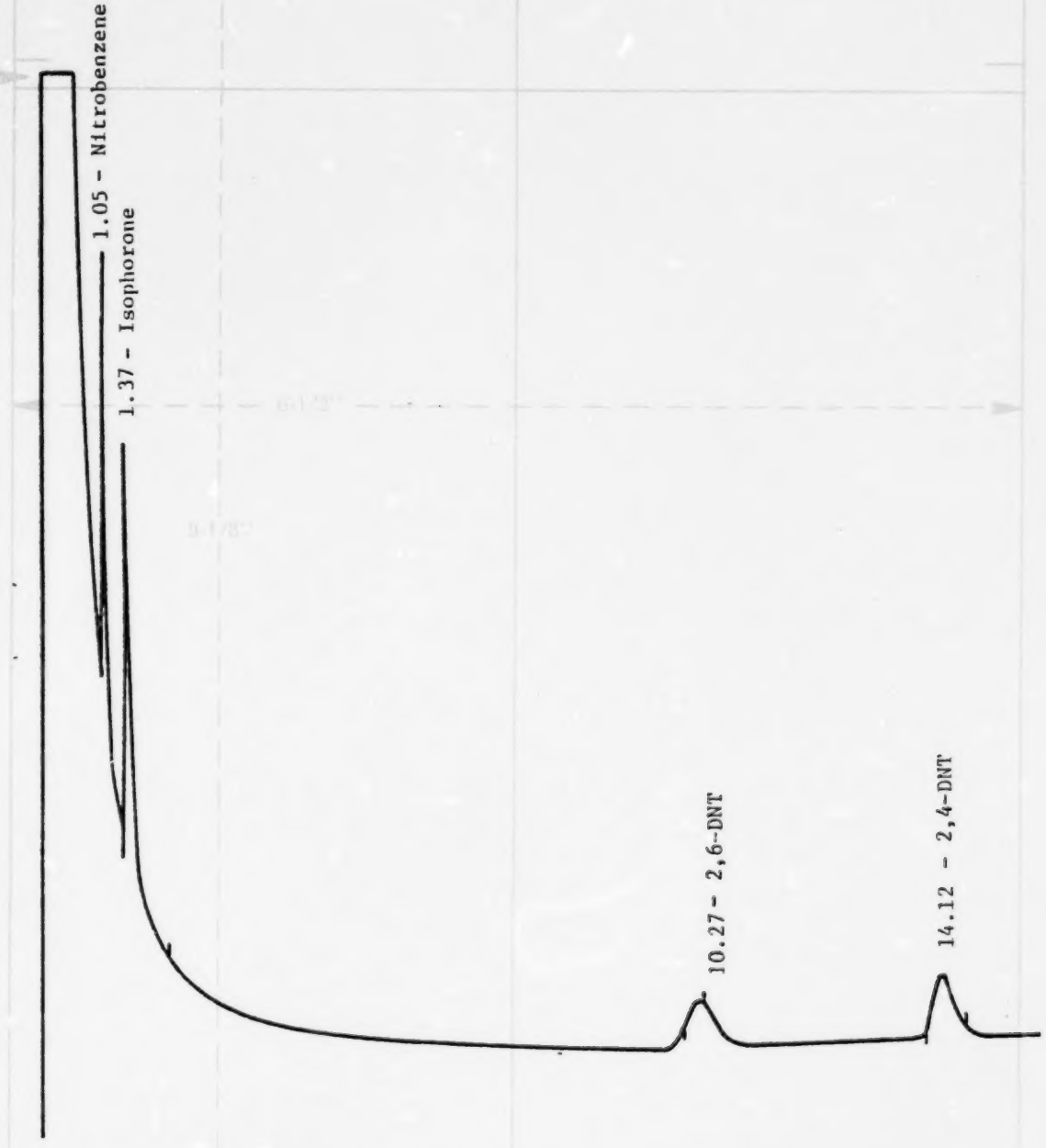

Figure 2. Chromatographic separation of the category 4 compounds at 120° on 1.95% QF 1/1.5% OV-17 at the 400 ng level.

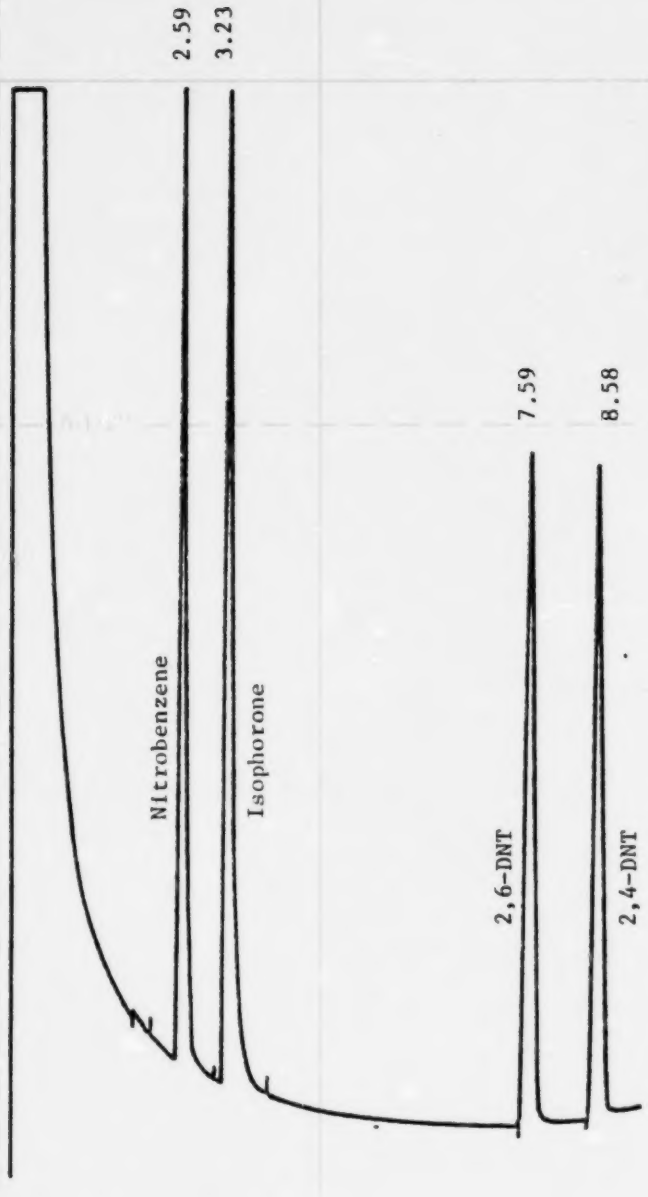

Figure 3. Chromatographic separation of the category 4 compounds at the 400 ng level on 1.95% QF-1/1.5% OV-17 using a temperature program from 80° to 170° at 10°/min.

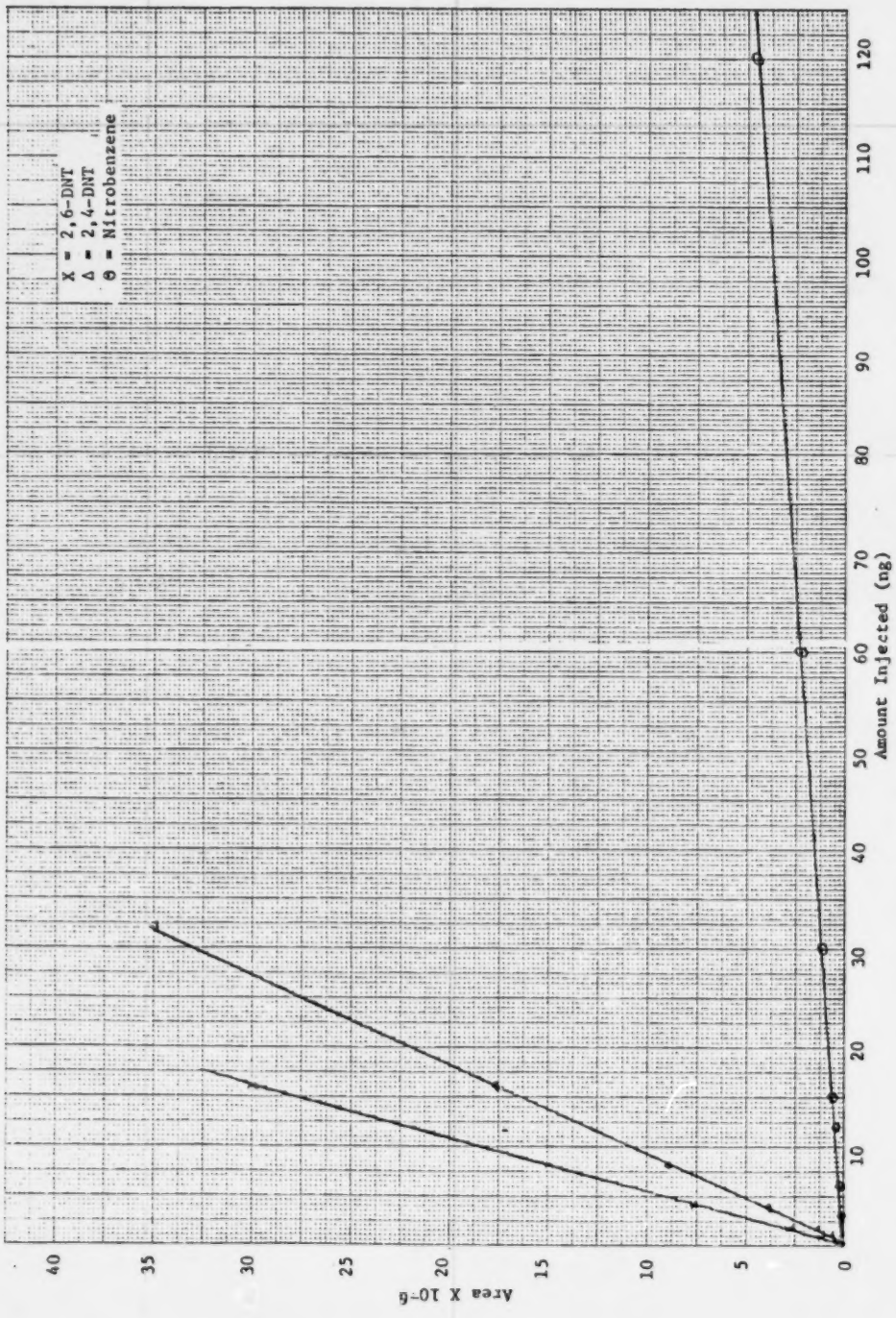

Figure 4. Response curve for nitroaromatics using GC/ECD.

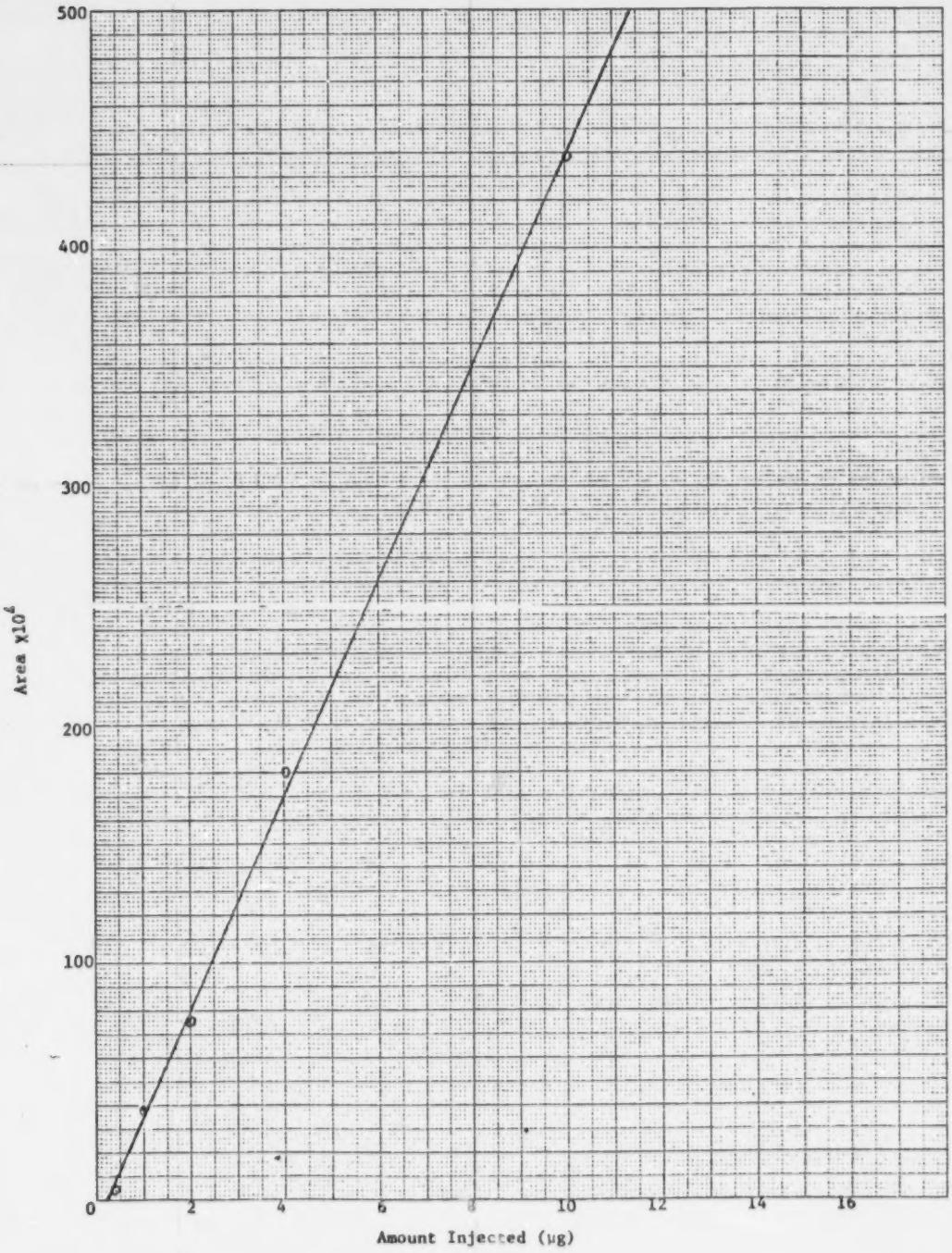

Figure 5. Response curve for isophorone using ECD.

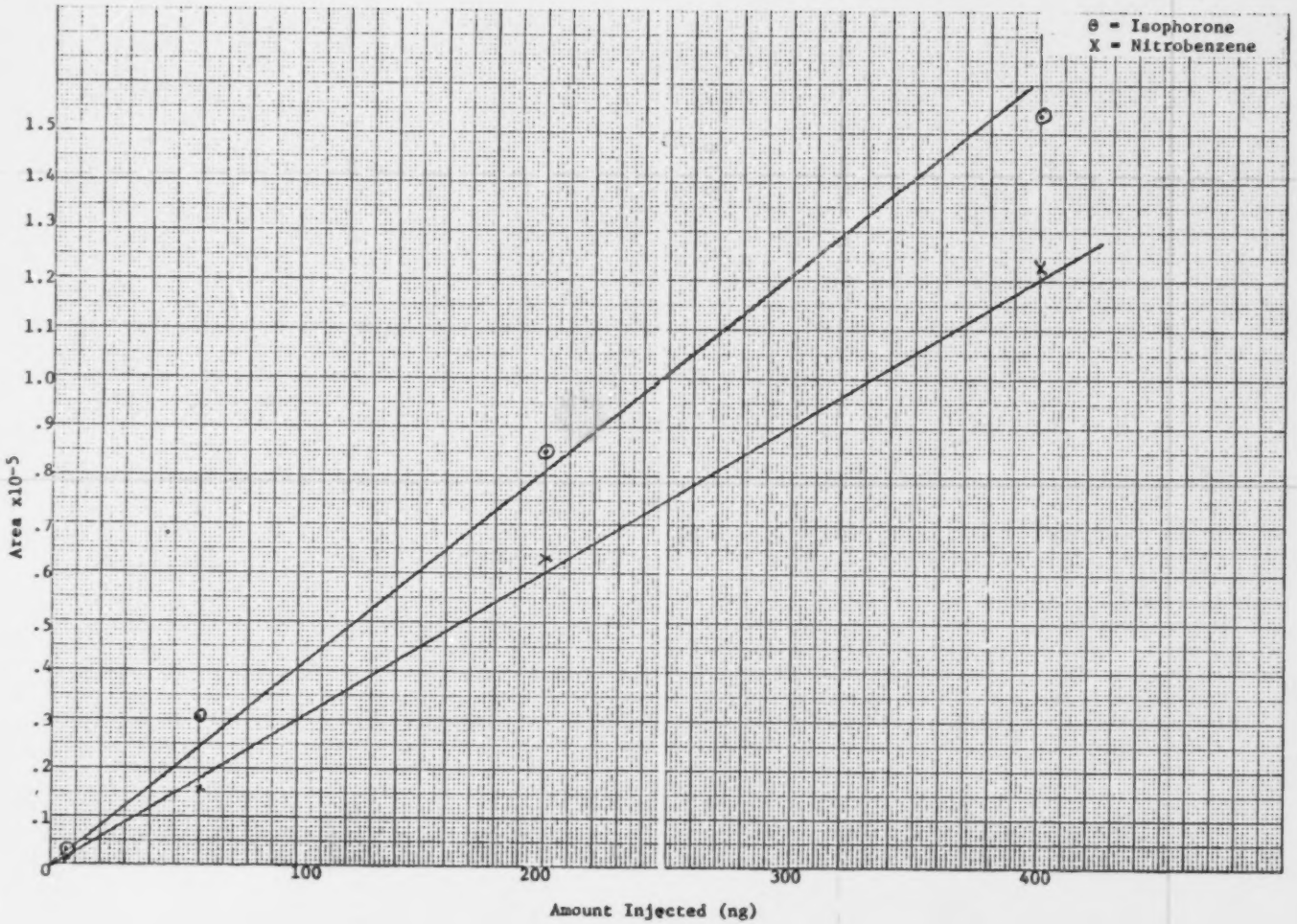

Figure 6. Response curve for nitrobenzene and isophorone using GC/FID.

was necessary either to select a temperature program condition or two different isothermal conditions for the determination of all four compounds. Figure 3 shows a temperature programmed run (80°-170° at 10°/min) which separates the four compounds quite nicely in a relatively short period of time (about 10 min). However, temperature programming cannot be done using many ECDs since a large increase in background level results, leading to decreased sensitivity. For this reason, two isothermal conditions were chosen (85°C for nitrobenzene and isophorone and 145°C for 2,4-DNT and 2,6-DNT) so that each of the components eluted within three to five minutes.

High Performance Liquid Chromatography (HPLC)

An evaluation was made of normal phase HPLC using ECD and UV detection, as described in the experimental section.

The noise level of the HPLC-ECD changed appreciably from day to day, possibly due to impurities in the solvents such as water or dissolved oxygen. Because of the difficulties involved with achieving a consistent, reproducible noise level from day to day, the detector was considered not to be desirable for this analysis. A chromatogram showing the separation and detection of the Category 4 compounds using HPLC-ECD is shown in Figure 7. The conditions used were those described in the Experimental Section.

A comparison of the UV and ECD sensitivities for the four compounds, as well as the GC-FID and GC-ECD detection limits is shown in Table 5.

TABLE 5. DETECTION LIMITS FOR NITROAROMATICS AND ISOPHORONE USING VARIOUS DETECTION SYSTEMS

	HPLC		GC	
	ECD	UV	FID	ECD
Nitrobenzene	22*	4.7	2.5	1.2
Isophorone	200	13.3	3.0	1.1
2,6-DNT	4	20.0	2.9	0.06
2,4-DNT	7	11.5	3.0	0.04

* Nanograms injected to produce 5 to 1 signal to noise ratio.

These data show that GC/ECD is far more sensitive than HPLC/ECD for all of these compounds. It is also apparent that HPLC/UV is nearly as sensitive as HPLC/ECD for these compounds. Based on this data it was concluded that GC/ECD or GC/FID would be a superior determination technique for these compounds.

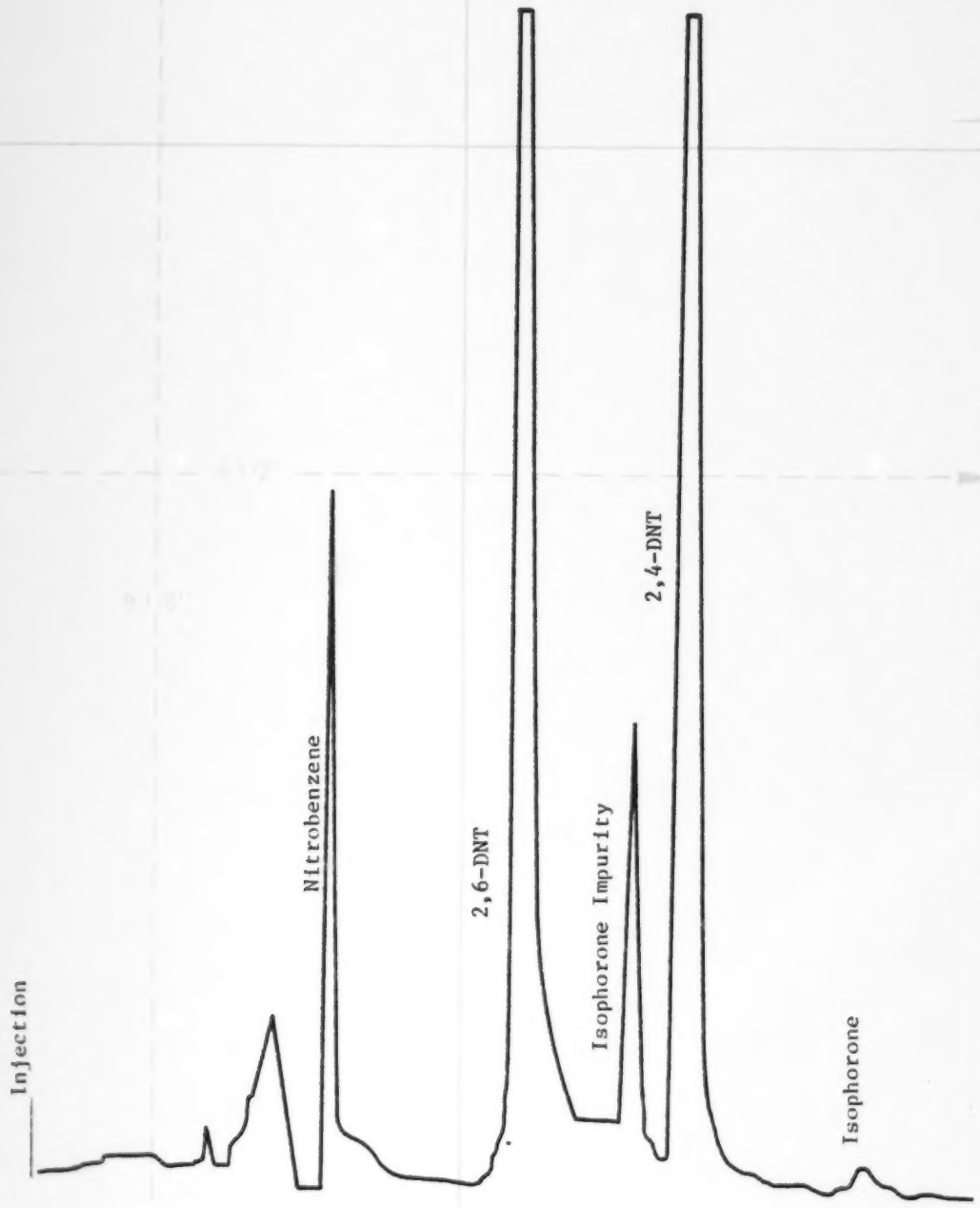

Figure 7. Chromatograph for HPLC separation of the nitroaromatic compounds using a Pye-Unican electron capture detector at the 200 ng level.

SOLVENT STABILITY STUDIES

The stabilities of the four compounds were found to be quite good in both acetone and methanol over the 90-day period, as shown in Tables 6 and 7. ANOVA analysis of the data, shown in Table 8, indicated that at the 95% confidence level (F.95) that there is a significant difference between the levels of isophorone, 2,4-DNT, and 2,6-DNT in methanol and 2,6-DNT in acetone with time. However, visual inspection of the data indicates that any difference which exists is quite small and the statistical significance is a result of the high precision of the assay and random bias of the chromatographic calibration from one time interval to the next.

The 95% confidence level was chosen since it represents a medium degree of precision whereas the 99% confidence level is relatively restrictive and the 90% confidence level is very non-restrictive. This choice is somewhat arbitrary and could be altered depending on the purpose of the particular study.

EXTRACTION STUDIES

The solvents methylene chloride and ethyl acetate were examined for the extraction of Category 4 compounds from aqueous solutions. Recoveries of all four of these compounds were greatest for methylene chloride at pH 7 and 10, as shown in Table 9. Visual inspection of the data in this table indicates a substantial decrease in recoveries at pH 2 in all cases. The effect of pH on recovery is much less obvious when the extraction is done with methylene chloride instead of ethyl acetate. ANOVA analysis of the data was conducted using a three way analysis program of the extraction efficiencies at each pH. This approach yields a single F value which points out whether or not there is a significant difference between any of the data sets, but does not decide which direction the difference is. The ANOVA data, shown in Table 10, indicates that there is a great dependence of recovery on extraction solvent, and thus methylene chloride is a significantly better solvent than ethyl acetate. The ANOVA also indicates that there is a significant dependence of extraction efficiency on pH for both solvents, and this dependence is much greater for ethyl acetate than methylene chloride. Visual inspection of the data shows that only the pH 2 data is lower using methylene chloride and these data points are undoubtedly the reason for the significant F value from ANOVA analysis.

In summary, the data shows that methylene chloride is a far better extracting solvent than ethyl acetate, for these compounds and that extraction at pH 7 or 10 with methylene chloride is more effective than extraction at pH 2.

As described in the experimental section, several related experiments were conducted to determine whether or not alternate work up procedures could be used effectively. The first such experiment examined whether: 1) 30 mL volumes of solvent could be used in place of 100 mL volumes and, 2) Na_2SO_4 could be used in place of $MgSO_4$. The results of this study are shown in Table 11. From these data, it is obvious that recoveries are not affected by using Na_2SO_4 in place of $MgSO_4$. In comparing these data

TABLE 6. SOLVENT STABILITY OF CATEGORY 4 COMPOUNDS IN ACETONE

Compound	Mean Concentration (ppm)	% Stability
Nitrobenzene		
Time 0 Days	200	100 ± 3.0
Time 30	202	101 ± 1.5
Time 60	199	100 ± 4.2
Time 90	197	99 ± 5.5
Isophorone		
Time 0	202	101 ± 4.0
Time 30	200	100 ± 3.5
Time 60	199	100 ± 4.7
Time 90	201	101 ± 1.7
2,6-DNT		
Time 0	199	100 ± 2.3
Time 30	203	102 ± 4.0
Time 60	194	97 ± 4.2
Time 90	196	98 ± 5.3
2,4-DNT		
Time 0	200	100 ± 2.0
Time 30	202	101 ± 3.5
Time 60	186	93 ± 1.7
Time 90	190	95 ± 3.1

TABLE 7. SOLVENT STABILITY OF CATEGORY 4 COMPOUNDS IN METHANOL

Compound	Mean Concentration (ppm)	% Stability
Nitrobenzene		
Time 0 Days	196	98 ± 3.5*
Time 30	196	98 ± 0.58
Time 60	197	99 ± 2.3
Time 90	201	101 ± 2.5
Isophorone		
Time 0	203	102 ± 0.58
Time 30	202	101 ± 0.58
Time 60	201	101 ± 1.7
Time 90	202	101 ± 2.1
2,6-DNT		
Time 0	200	100 ± 4.9
Time 30	182	91 ± 0.58
Time 60	197	99 ± 2.5
Time 90	209	105 ± 2.5
2,4-DNT		
Time 0	201	101 ± 6.5
Time 30	206	100 ± 2.0
Time 60	184	92 ± 2.6
Time 90	193	97 ± 2.3

*Standard deviation for triplicate analysis.

TABLE 8. ANOVA ANALYSIS OF SOLVENT STABILITY DATA

	Fexp acetone	Fexp methanol
Nitrobenzene	0.466	2.93
Isophorone	0.525	186*
2,6-DNT	6.95*	14.1*
2,4-DNT	3.79	23.1*
*F.99 = 7.59	4.59	4.07

*Exceeds F.95 Test for significance.

TABLE 9. RECOVERY FROM TRIPLICATION EXTRACTION OF WATER DOSED AT 40 PPB LEVEL

Compound	pH	Solvents	
		Methylene chloride	Ethyl acetate
Nitrobenzene			
	2	81.0 ± 7.5*	34.9 ± 7.6
	7	103.0 ± 2.4	83.0 ± 4.8
	10	101.0 ± 3.7	65.0 ± 7.8
Isophorone			
	2	80.0 ± 8.0	39.2 ± 9.2
	7	107.0 ± 4.9	83.0 ± 3.3
	10	104.0 ± 3.9	60.0 ± 2.9
2,6-DNT			
	2	71.0 ± 10.0	24.0 ± 12.0
	7	88.0 ± 8.2	72.0 ± 1.8
	10	89.0 ± 0.39	65.1 ± 3.4
2,4-DNT			
	2	69.2 ± 16.0	27.0 ± 14.0
	7	90.0 ± 10.0	70.0 ± 2.2
	10	91.0 ± 1.7	75.0 ± 4.9

*% recovery ± standard deviation

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TABLE 10. ANOVA ANALYSIS OF EXTRACTION DATA

I) F_{exp} values for the effect of change in pH for each extraction solvent.

Compound	Methylene chloride	Ethyl acetate
Nitrobenzene	23.1*	109*
Isophorone	23.0*	182*
2,6-DNT	8.75*	414*
2,4-DNT	6.51*	207*
F.95	5.14	5.14

II) F_{exp} values for the effect of extraction solvent at constant pH.

Compound	pH 2	pH 7	pH 10
Nitrobenzene	143*	55.8*	97.4*
Isophorone	92*	48.6*	298*
2,6-DNT	109*	14.3*	337*
2,4-DNT	39*	13.9*	47.6*
F.95	7.71	7.71	7.71

*Significant difference at F.95.

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TABLE 11. RECOVERY DATA FOR EFFECT OF DRYING AGENT AND EXTRACTION SOLVENT VOLUME

Compound / Extraction Solvent / Drying Agent	Recovery: 100 mL $MeCl_2$* / $MgSO_4$	Recovery: 30 mL $MeCl_2$ / Na_2SO_4	Recovery: 30 mL $MeCl_2$ / $MgSO_4$
Nitrobenzene	103 ± 2.4**	83 ± 2.0	81 ± 3.3
Isophorone	107 ± 4.9	87 ± 1.5	86 ± 2.0
2,6-DNT	88 ± 8.2	94 ± 2.0	98 ± 13.0
2,4-DNT	90 ± 10.0	85 ± 3.0	84 ± 3.5

* Data from Table 9.
** Percent recovery ± standard deviation for triplicate extractions at the 40 ppb level and pH 7.

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to those in Table 9 for pH 7 and methylene chloride, it can be seen that a slight decrease in recovery is noted for some of the compound using 30 mL rather than 100 mL quantities of solvent. However, since the recoveries are still quite good (> 85%) using 30 mL volumes it was felt that this volume could be used in the extraction protocol.

A second related experiment was conducted in which K-D concentration was compared to vortex concentration, since many laboratories do not have a vortex evaporator. The data for this experiment are given in Table 12. From these data it is obvious that the K-D and vortex concentration are equally effective and thus either one could be used in the protocol.

A third experiment was conducted to determine whether or not significant loss occurs when methylene chloride is displaced with toluene, using the K-D concentrator. When a methylene chloride solution was concentrated and solvent displaced with toluene recoveries were 93 ± 1.2% for nitrobenzene, 94 ± 1.3% for isophorone, 100 ± 2.2% for 2,6-DNT, and 102 ± 3.1% for 2,4-DNT. Thus, no appreciable losses occurred during solvent exchange and this protocol can be followed when using the GC-ECD, which is incompatible with methylene chloride.

Based on these studies, the preferred extraction protocol is as follows: 1) solvent extract with 3 x 60 mL/liter of sample of methylene chloride at pH 7; 2) dry extract by filtration through 2 grams of Na_2SO_4 or $MgSO_4$; and 3) concentrate the extract to about 1 mL using K-D concentration. If GC-ECD is to be used the extract must be solvent exchanged with toluene by addition of 1 mL of toluene and reconcentration of the extract to 1 mL using K-D concentration.

PRESERVATION STUDIES

The stability data for this study are presented in Tables 13 and 14. The stabilities presented were not corrected for extraction efficiency since 1) quantitative (> 90%) extraction was observed at pH 7 and pH 10 using methylene chloride; and 2) although the extraction efficiencies at pH 2, from the previous study, were on the order of 80%, the extraction of the stored water samples had apparently higher recoveries, for an as yet unknown reason.

Stabilities of the compounds appeared to be highest at 4°C and pH 7 or pH 2 in the absence of chlorine as shown in Tables 13 and 14. ANOVA analysis, as shown in Table 15, indicates that some small variations in the recoveries may be attributed to chlorine content in the samples, however, visual inspection of the data shows the effect of added chlorine to be inconsistent and quite small.

On the basis of this data the suggested storage condition is pH 7 and 4°C. Storage at 4°C is preferred since biological degradation, which could occur in actual samples, will be retarded under reduced temperature conditions.

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TABLE 12. COMPARISON OF K-D AND VORTEX CONCENTRATION

Compound	Recovery	
	K-D	Vortex
Nitrobenzene	88 ± 4.7*	89 ± 2.9
Isophorone	88 ± 4.9	93 ± 2.0
2,6-DNT	89 ± 4.3	93 ± 2.7
2,4-DNT	89 ± 5.7	94 ± 2.7

*Percent recovery ± standard deviation for triplicate analysis.

TABLE 13. PERCENT RECOVERIES OF CATEGORY NO. 4 COMPOUNDS STORED IN DUPLICATE AT THE 40 PPB LEVEL AND ROOM TEMPERATURE

Room temperature	No chlorine	2 ppm chlorine
pH 2		
Nitrobenzene	85.8 ± 7.6	83.7 ± 1.4
Isophorone	87.0 ± 13.0	82.5 ± 1.2
2,6-Dinitrotoluene	96.0 ± 10.0	92.3 ± 2.6
2,4-Dinitrotoluene	96.5 ± 16.0	90.5 ± 0.6
pH 7		
Nitrobenzene	72.9 ± 11.0	73.8 ± 8.4
Isophorone	73.4 ± 13.0	74.5 ± 7.8
2,6-Dinitrotoluene	81.0 ± 11.0	81.5 ± 7.9
2,4-Dinitrotoluene	76.3 ± 21.0	86.7 ± 8.5
pH 10		
Nitrobenzene	77.4 ± 2.0	67.7 ± 0.91
Isophorone	78.8 ± 2.0	72.0 ± 0.0
2,6-Dinitrotoluene	83.3 ± 1.2	78.3 ± 0.0
2,4-Dinitrotoluene	82.7 ± 2.5	82.9 ± 0.0

TABLE 14. PERCENT RECOVERIES OF CATEGORY 4 COMPOUNDS STORED IN DUPLICATE AT THE 40 PPB LEVEL AND 4°C

4°C	No chlorine	2 ppm chlorine
pH 2		
Nitrobenzene	87.7 ± 0.071*	67.9 ± 4.2
Isophorone	96.2 ± 0.61	74.0 ± 4.7
2,6-DNT	105.0 ± 4.9	91.3 ± 11.0
2,4-DNT	99.8 ± 8.8	85.6 ± 9.8
pH 7		
Nitrobenzene	86.4 ± 2.4	79.1 ± 23.0
Isophorone	88.0 ± 1.9	79.5 ± 22.0
2,6-DNT	97.4 ± 3.3	90.0 ± 23.0
2,4-DNT	105.0 ± 4.2	83.0 ± 9.0
pH 10		
Nitrobenzene	77.9 ± 12.6	51.9 ± 19.0
Isophorone	81.4 ± 13.2	53.6 ± 17.0
2,6-DNT	91.8 ± 20.0	64.4 ± 12.0
2,4-DNT	83.8 ± 10.4	62.2 ± 8.6

* Percent recovery ± standard deviation.

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TABLE 15. TABLE OF F VALUES FROM ANOVA ANALYSIS OF PRESERVATION DATA

ANOVA by temperature

	No chlorine	2 ppm chlorine
pH 2		
Nitrobenzene	0.126	25.0*
Isophorone	0.936	6.30
2,6-DNT	1.18	0.017
2,4-DNT	0.062	0.500
F.95	10.1	10.1
pH 7		
Nitrobenzene	2.69	0.095
Isophorone	2.48	0.092
2,6-DNT	3.98	0.256
2,4-DNT	3.48	0.181
F.95	10.1	10.1
pH 10		
Nitrobenzene	0.003	0.890
Isophorone	0.076	2.36
2,6-DNT	0.360	2.75
2,4-DNT	0.023	11.5*
F.95	10.1	10.1

ANOVA by pH

	Room Temperature		$4^{o}C$	
	No Chlorine	Chlorine	No Chlorine	Chlorine
Nitrobenzene	1.35	7.32	1.02	1.25
Isophorone	0.805	2.88	1.83	1.41
2,6-DNT	1.74	4.68	0.553	1.82
2,4-DNT	0.887	1.19	3.59	3.91
F.95	9.55	9.55	9.55	9.55

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TABLE 15. (continued)

ANOVA by chlorine content

	Room temperature		4°C	
	No chlorine	2 ppm chlorine	No chlorine	2 ppm chlorine
pH 2				
Nitrobenzene	0.356	0.156	0.595	43.8*
Isophorone	6.95	0.225	1.01	44.6*
2,6-DNT	2.09	0.254	4.24	2.55
2,4-DNT	3.76	0.274	0.016	2.31
F.95	10.1	10.1	10.1	10.1
pH 7				
Nitrobenzene	0.00	0.008	1.49	0.209
Isophorone	0.001	0.011	1.12	0.297
2,6-DNT	0.006	0.003	0.200	0.211
2,4-DNT	0.376	0.413	0.118	9.69
F.95	10.1	10.1	10.1	10.1
pH 10				
Nitrobenzene	0.234	81.3*	2.17	2.56
Isophorone	7.44	22.3*	3.61	3.36
2,6-DNT	4.05	35.3*	4.77	2.78
2,4-DNT	6.35	0.120	16.7*	5.14
F.95	10.1	10.1	10.1*	10.1

* Significant difference at F .95.

CLEAN UP METHOD EVALUATION

The results of this study are presented in Table 16. The compounds of interest eluted in several different fractions on silica gel, but in a single fraction on Florisil (conditions 1 and 2 in Table 15 respectively). Therefore, Florisil was considered most appropriate for this application.

Since acetone boils at a somewhat lower temperature than methanol, it was considered useful to investigate the elution of the Category 4 compounds using acetone as a mobile phase modifier. This scheme is shown in Table 16 as condition 3. As shown in Table 16, the compounds eluted primarily in the 40% and 50% acetone/$MeCl_2$ fractions. This scheme, in which fractions 2-4 were collected, was used for the clean up of the first three wastewater samples described in the next section. For the last three water samples condition 4, Table 16, was used as described in the following section. However, it was discovered in subsequent work that in some circumstances the nitrotoluenes partially elute in the methylene chloride fraction. For this reason the final protocol, in the Appendix, has been modified to include the use of 10% $MeCl_2$ in hexane, rather than 100% $MeCl_2$ in the initial eluate.

WASTEWATER ANALYSIS

The procedure presented in the Appendix was applied to a series of six water samples representing surface water, municipal effluents, and several industrial effluent types. As described in the experimental section, the clean up and determinative methods were revised after the analysis of several of the wastewaters. All samples were subjected to clean up before analysis. A description of the problems associated with the analysis of each sample follows. All data obtained from the analysis of these samples is given in Table 17.

Sample 1 was a sample drawn from a pump receiving wastes from various process areas, including phenolic resins, vinyl acetate, and polyvinyl chloride. The sample appeared to contain about 40 ppb of isophorone and about 0.2 ppb of 2,6-DNT, as shown in Figure 8 and Table 17. Although it did not appear to contain detectable levels of nitrobenzene or 2,4-DNT, there were two peaks, one eluting slightly before and one eluting slightly after 2,4-DNT, thus increasing its MDL. Recoveries of the spiked compounds were relatively good, although only about 50% recoveries were realized for 2,4- and 2,6-DNT. Upon storage for seven days at 4°C the isophorone appeared to have decomposed whereas the other components had not (to a great extent). Based on visual inspection of the unspiked chromatograms and the recoveries for spiked compounds the estimated MDLs in this sample are 2 ppb for nitrobenzene, 25 ppb for isophorone, 0.1 for 2,6-DNT, and 0.4 for 2,4-DNT. On inspection of the spiked sample chromatogram (Figure 9) for 2,4-DNT it was noted that the GC integration system integrated only one peak for the spiked sample, which is a composite of the 2,6-DNT and the two overlapping peaks mentioned previously (and shown in Figure 8). In order to obtain a reasonably accurate area for 2,4-DNT alone, the areas for the two peaks from the unspiked samples were subtracted from the area of the single peak for the spiked sample. This approach is, of course, not useable in obtaining data from unspiked samples.

Condition	Stationary phase	Mobile phase composition	% recovery			
		10 mL each	Nitrobenzene	Isophorone	2,6-DNT	2,4-DNT
1	Silica Gel	1. Pentane	ND*	ND	ND	ND
		2. 5% $MeCl_2$/Pentane	ND	ND	ND	ND
		3. 20% $MeCl_2$/Pentane	ND	ND	ND	ND
		4. 50% $MeCl_2$/Pentane	ND	ND	ND	ND
		5. 100% $MeCl_2$	77.7 ± 4.0**	ND	35.6 ± 3.7	15.4 ± 2.3
		6. 20% MeOH/$MeCl_2$	ND	ND	41.4 ± 4.5	61.7 ± 1.8
		7. 50% MeOH/$MeCl_2$	ND	68.4 ± 2.5	3.0 ± 1.7	6.9 ± 2.8
		8. 50% NeOH/$MeCl_2$	ND	ND	ND	ND
		9. 50% MeOH/$MeCl_2$	ND	ND	ND	ND
	TOTAL		77.7 ± 4.0	68.4 ± 2.5	80.0 ± 3.3	84.0 ± 2.3
2	Florisil 120°C activation	Same as condition 1. Only fraction 7 contained detectable amounts of the compounds.	74.6 ± 3.2	75.2 ± 4.2	74.1 ± 4.5	77.0 ± 3.9
	TOTAL		74.6 ± 3.2	75.2 ± 4.2	74.1 ± 4.5	77.0 ± 3.9
		10 mL each				
3	Florisil 120°C activation	1. 30% Ac/$MeCl_2$	ND	ND	ND	ND
		2. 40% Ac/$MeCl_2$	18.2 ± 2.5	6.5 ± 3.0	21.2 ± 2.7	17.8 ± 2.8
		3. 50% Ac/$MeCl_2$	52.4 ± 3.3	65.0 ± 2.7	52.9 ± 1.9	56.7 ± 3.1
		4. 60% Ac/$MeCl_2$	0.94 ± 2.5	3.2 ± 1.7	1.7 ± 2.5	1.8 ± 2.9
		5. 70% Ac/$MeCl_2$	ND	ND	ND	ND
		6. 80% Ac/$MeCl_2$	ND	ND	ND	ND
	TOTAL		71.5 ± 2.8	74.7 ± 2.5	75.8 ± 2.4	76.3 ± 2.9
		30 mL each				
4	Florisil 200°C activation	1. 100% $MeCl_2$	ND	ND	ND	ND
		2. 10% Ac/$MeCl_2$	97.0 ± 2.8	99.0 ± 1.0	97.0 ± 4.5	96.0 ± 4.6
		3. 10% Ac/$MeCl_2$	ND	ND	ND	ND

* Detection limit corresponds to ~1% recovery.
** Standard deviation for triplicate runs.

TABLE 16. COLUMN CHROMATOGRAPHY DATA

Sample No.	Sample type	Samples background level (ppb)		Level spiked (ppb)	% Recovery 0 days	% Recovery 7 days at 4°C
1	Sewer pump receiving wastes from various process areas.	Nitrobenzene	< 2	13	124 ± 12(1)	86 ± 16
		Isophorone	40.5 ± 32.0	270	67 ± 30	< 5
		2,6-DNT	0.25 ± 0.01	0.9	58 ± 15	53 ± 19
		2,4-DNT	< 0.4 ± (2)	0.9	47 ± 18	42 ± 18
2	Brine sample from holding tank receiving washings from ships carrying various commodities.	Nitrobenzene	< 1	13	67 ± 40	130 ± 15
		Isophorone	< 50	270	110 ± 44	100 ± 54
		2,6-DNT	< 0.1	0.9	12 ± 6	4 ± 2.5
		2,4-DNT	< 1	0.9	4 ± 4	5 ± 3
3	Secondary sewage effluent (Columbus, Ohio).	Nitrobenzene	< 2	96	84 ± 8	77 ± 1
		Isophorone	120 ± 6	560	87 ± 5	78 ± 2
		2,6-DNT	< 0.1	7	87 ± 8	88 ± 2
		2,4-DNT	< 0.1	8	79 ± 6	72 ± 4
4	Final effluent from UNOX treatment system receiving wastes from various chemical plants.	Nitrobenzene	10 ± 3	96	70 ± 6	71 ± 3
		Isophorone	< 5	560	71 ± 5	77 ± 2
		2,6-DNT	< 0.1	17	78 ± 1	78 ± 1
		2,4-DNT	< 0.1	18	70 ± 2	70 ± 2
5	Surface water (Olentangy River).	Nitrobenzene	< 5	90	70 ± 6	71 ± 3
		Isophorone	< 5	50	71 ± 5	77 ± 2
		2,6-DNT	< 0.05	5	78 ± 2	78 ± 1
		2,4-DNT	< 0.05	5	70 ± 2	70 ± 1
6	Final effluent from a chemical manufacturing plant producing nitrobenzene, O-dichlorobenzene, O-nitrophenol, aniline, and various oil additives.	Nitrobenzene	27 ± 10	100	35 ± 11	93 ± 2
		Isophorone	< 20	100	----	51 ± 8
		2,6-DNT	^5	50	13 ± 2	25 ± 2
		2,4-DNT	< 20	100	8 ± 2	----

(1) Average recovery and standard deviation.

TABLE 17. ANALYTICAL RESULTS FROM VARIOUS WASTEWATER SAMPLES

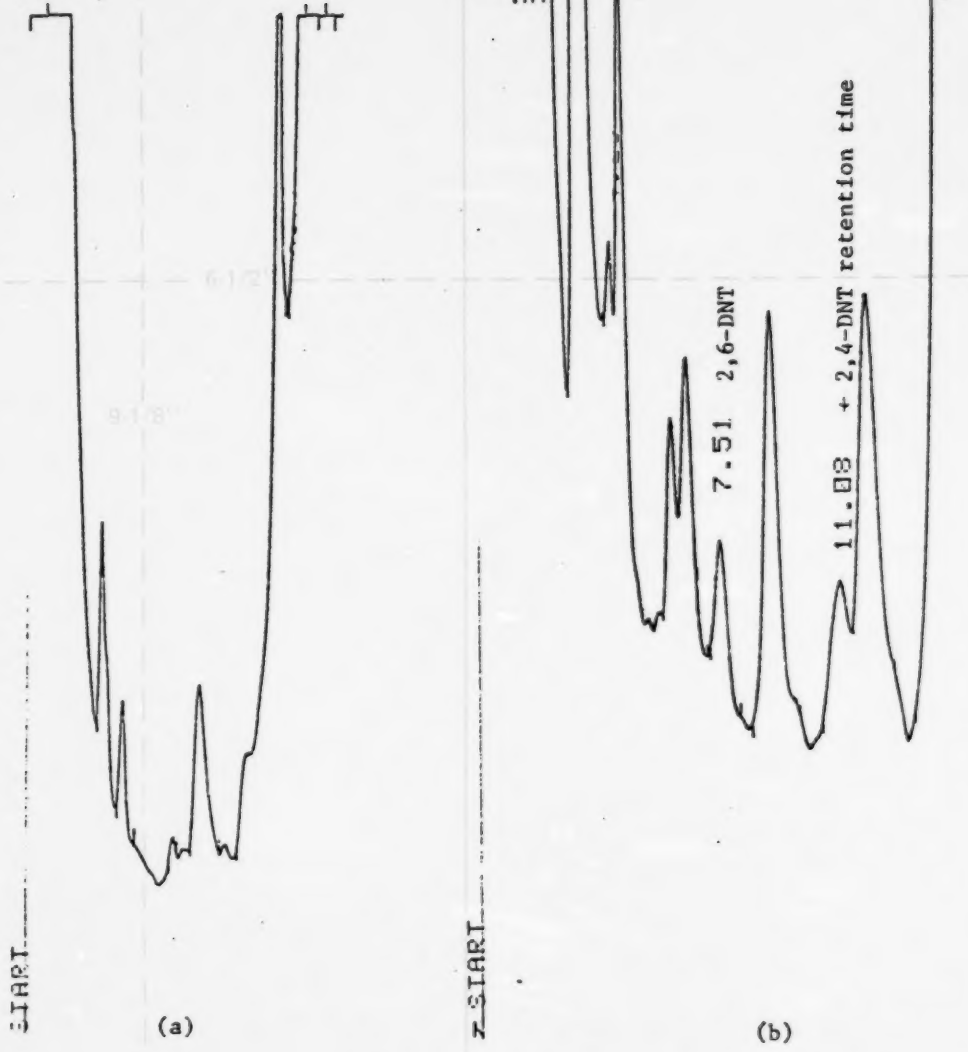

Figure 8. Chromatograms for Sample 1, unspiked, using ECD at 85°C, Isothermal (a), and ECD at 120°C Isothermal.

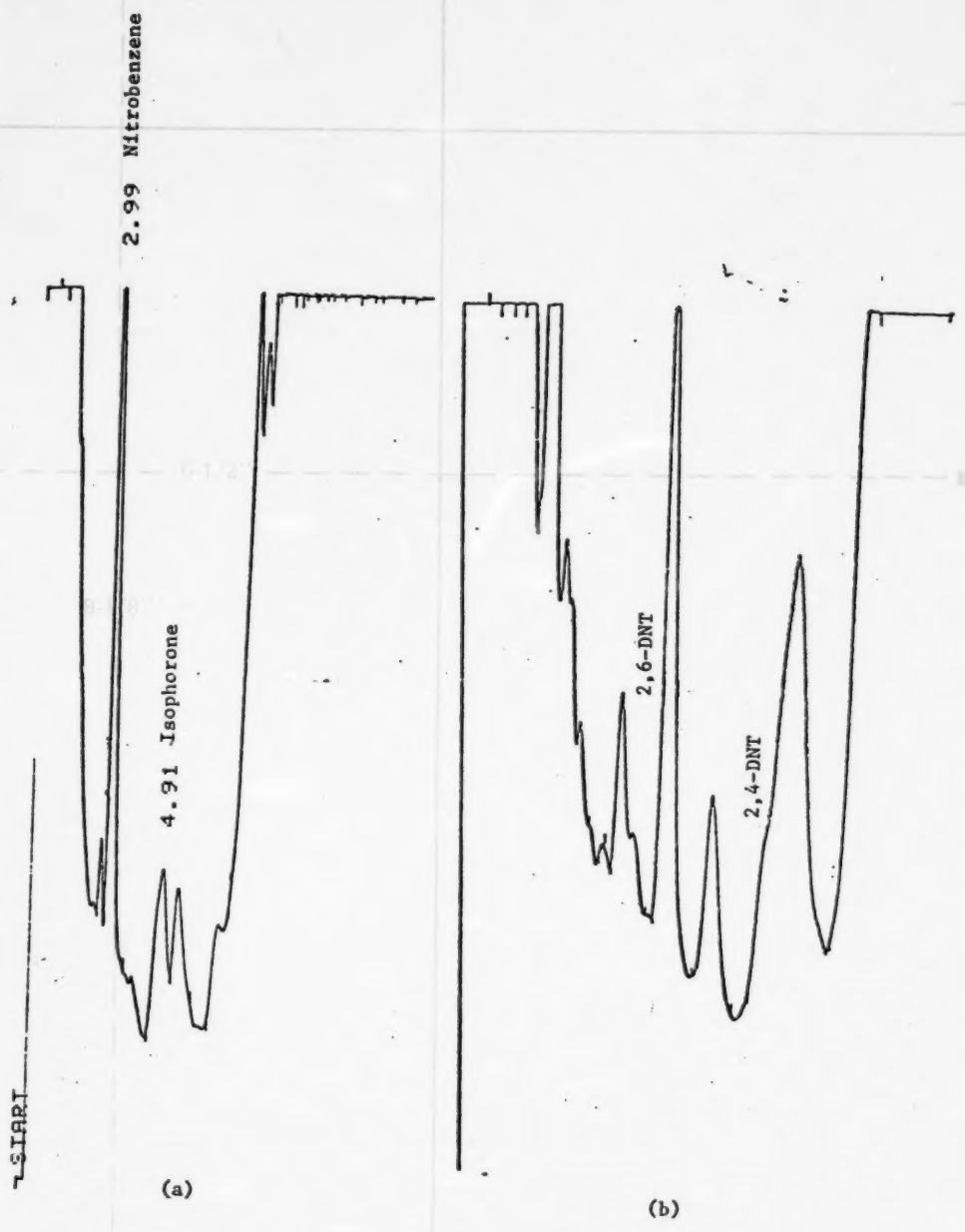

Figure 9. Chromatograms for Sample 1, spiked with 13 ppb of nitrobenzene, 270 ppb of isophorone, and 0.88 ppb each of 2,4- and 2,6-DNT, using ECD at 85°C (a), and 120°C (b).

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Sample 2 was a seawater sample from a holding tank receiving washings from ships hauling various commodities, and this holding tank had been determined to contain isophorone on a previous sampling visit. Chromatographs for this sample, unspiked and spiked, are shown in Figures 10 and 11 respectively.

This sample was difficult to analyze since substantial variations in background level were observed for replicate extractions. None of the components of interest were detected in the sample, based on retention time data. However the MDLs for the components in this sample were considerably higher than that observed for distilled water blanks or other wastewater samples.

Recoveries of the compounds spiked into sample 2 were apparently satisfactory for nitrobenzene and isophorone, although precise quantitation was not possible due to wide variations in background. However, recoveries of 2,4- and 2,6-DNT were very low. Based on the background levels in the sample and the recoveries observed, the MDLs are estimated to be 2 ppb for nitrobenzene, 50 ppb for isophorone, and 1 ppb for 2,4-DNT, and 2,6-DNT.

Sample 3 was a secondary sewage effluent and was the first sample processed using the modified clean up method (condition 4, Table 2). The chromatograms for the unspiked and spiked samples are shown in Figures 12 and 13 respectively. The unspiked sample did not contain detectable levels of 2,4- or 2,6-DNT or nitrobenzene, but did appear to contain about 100 ppb of isophorone, based on retention time data. The extraction efficiency and storage stability were > 70% for all components as shown in Table 17. Based on the background level observed for the unspiked sample MDLs are estimated to be 2 ppb for nitrobenzene, 40 ppb for isophorone, and 0.05 ppb for 2,4- and 2,6-DNT.

The only disturbing aspect concerning the analysis of this sample is the apparently high background for isophorone. It is possible that due to this rather low electron capture response, a small quantity of a highly electrocapturing species might have been mistaken as a large quantity of isophorone. For this reason, FID was employed for all future isophorone determinations.

Sample 4 was a final effluent from a UNOX treatment system receiving wastes from plants producing plasticizers, butyl rubber, and olefins. This was the first sample where FID, rather than ECD, was used to determine nitrobenzene and isophorone. This approach was taken since the ECD background from all the previous samples had limited the sensitivity for isophorone to about 40 ppb. Nitrobenzene was also determined by FID. To measure it by ECD would require an additional chromatographic run. However, if one desired only to measure nitrobenzene, ECD probably would be most effective, based on the background levels for the samples evaluated herein. Chromatograms for sample 4 unspiked and spiked are shown in Figures 14 and 15, respectively. For 2,4- and 2,6-DNT as well as for isophorone, no detectable quantities were found, whereas a small quantity (about 10 ppb) of nitrobenzene and isophorone appeared to be present, based solely on retention time data. Recoveries from the spiked sample were > 70% for all components both before and after seven days' storage at 4°C. Based on the

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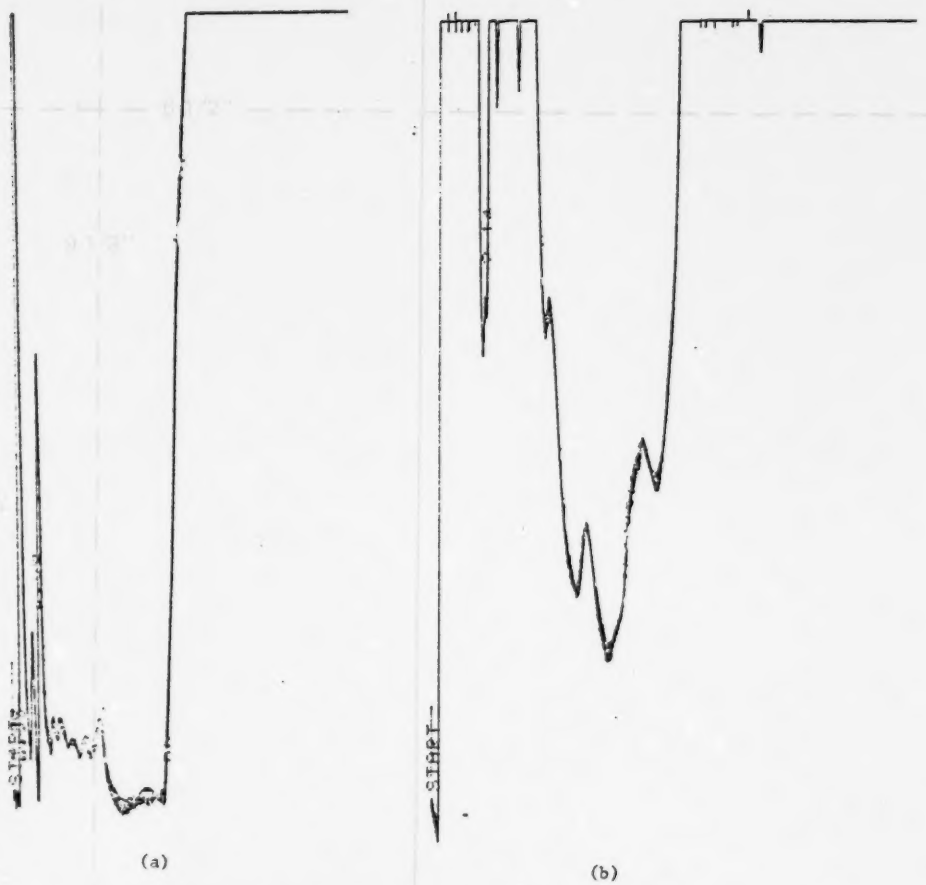


(a) (b)

Figure 10. Chromatograms for Sample 2, unspiked using ECD at 85°C (a), and 170°C (b).

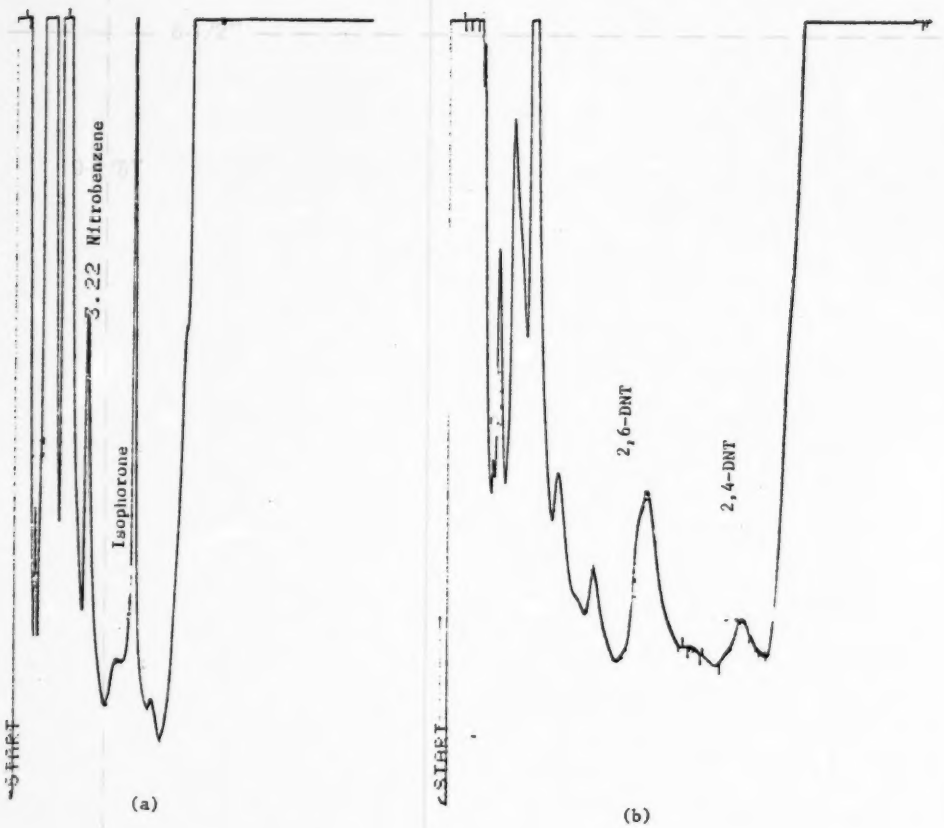


Figure 11. Chromatograms for Sample 2, spiked with 13 ppb nitrobenzene, 270 ppb isophorone, and 0.88 ppb of 2,4- and 2,6-DNT, using ECD at 85°C (a) and 120°C (b).

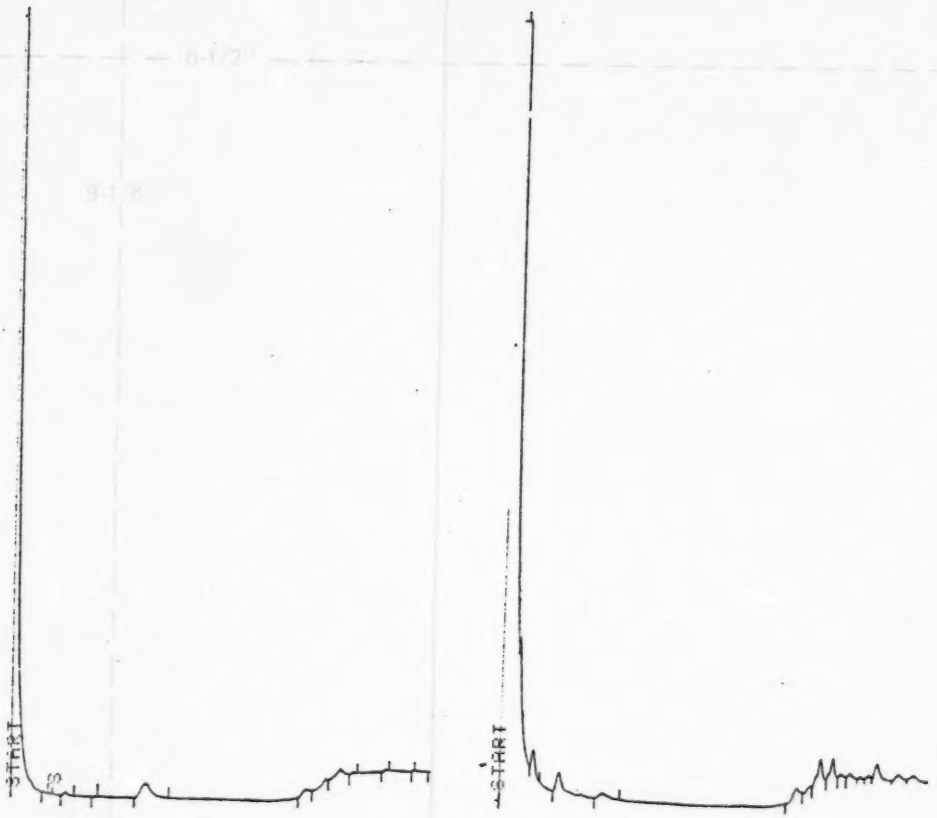


Figure 12. Chromatograms for Sample 3, unspiked, using ECD at 85°C (a) and 145°C (b).

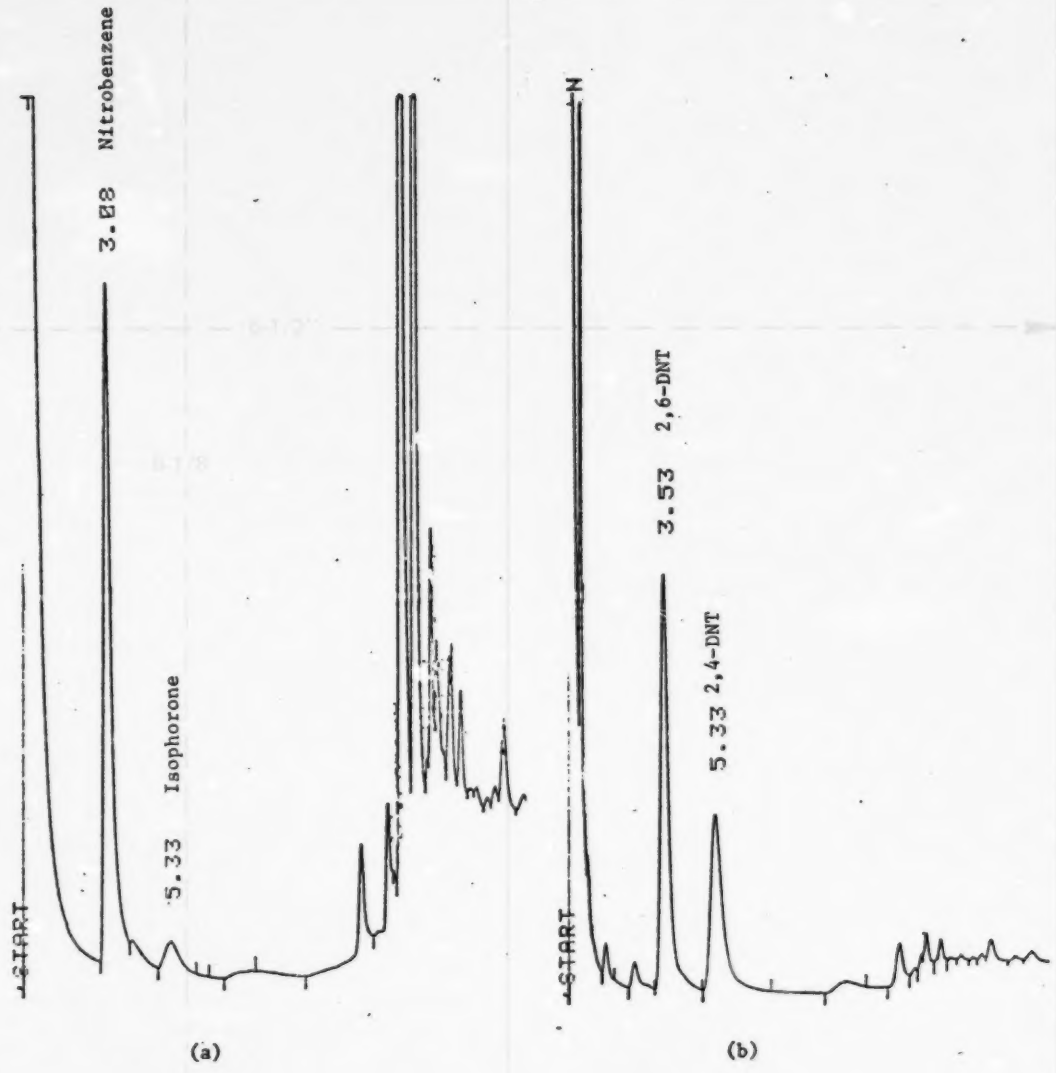
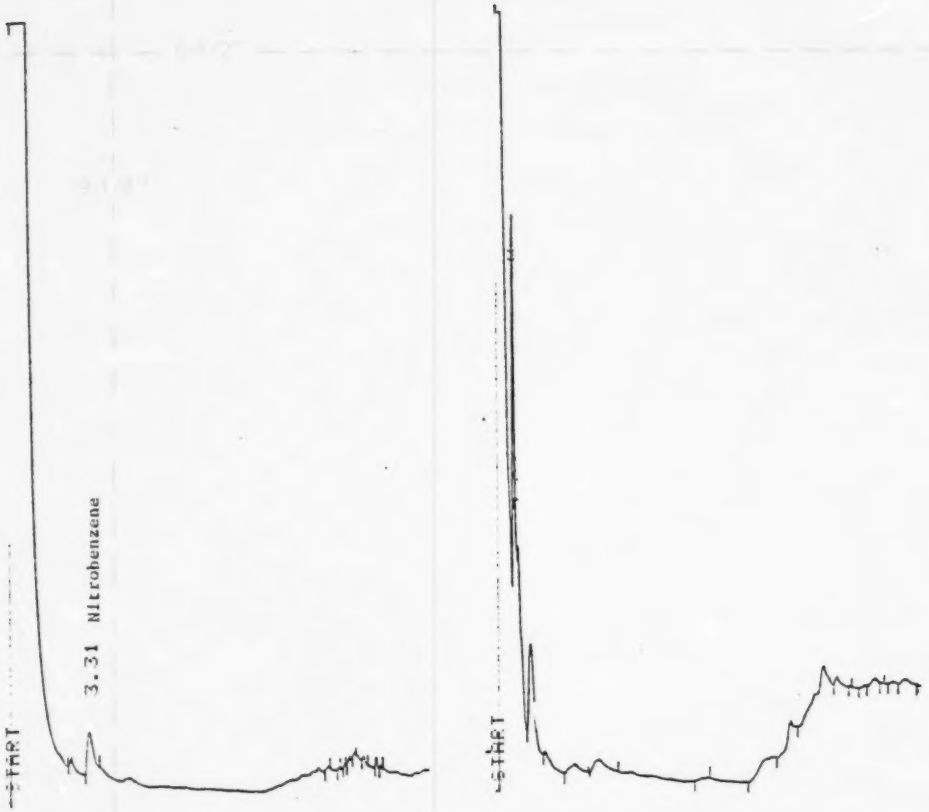


Figure 13. Chromatograms for Sample 3, spiked with 96 ppb of nitrobenzene, 560 ppb of isophorone, 70 ppb of 2,6-DNT, and 8 ppb of 2,4-DNT, using ECD at 85°C (a) and 145°C (b).



(a) (b)

Figure 14. Chromatograms for Sample 4, unspiked using FID at 85°C (a), and ECD at 145°C (b).

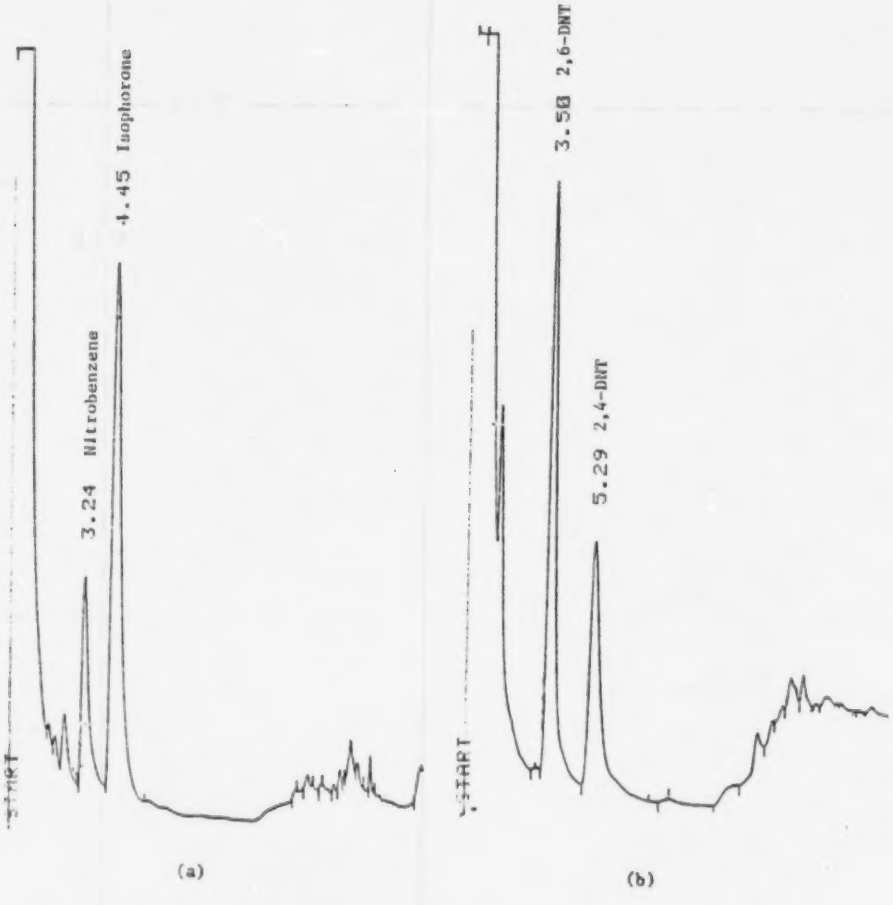


Figure 15. Chromatogram for Sample 4, spiked with 96 ppb nitrobenzene, 560 ppb isophorone, 7 ppb of 2,6-DNT, and 8 ppb of 2,4-DNT, using FID at 85°C (a), and ECD at 145°C (b).

background level and recovery data the MDLs in this sample are estimated to be .1 ppb for 2,4- and 2,6-DNT, and 5 ppb for nitrobenzene and isophorone, based on a five to one signal to noise ratio.

Sample 5 was a surface water sample and contained no detectable levels of any of the four compounds. Chromatograms for the unspiked and spiked samples are shown in Figures 16 and 17 respectively. Recoveries were > 70% for all components both before and after storage for seven days at 4°C. The estimated MDLs for this sample are 5 ppb for isophorone and nitrobenzene, and 0.05 ppb for 2,4- and 2,6-DNT.

Sample 6 was a final effluent from a chemicals plant producing a variety of chemicals including nitrobenzene, o-dichlorobenzene, aniline, nitrophenol, and various oil additives. For this sample nitrobenzene and isophorone were determined by FID and 2,4- and 2,6-DNT were determined by ECD. The overall background level of this sample was rather high, and was not appreciably reduced using the florisil clean up procedure. A small but distinct peak was observed in the unspiked extract corresponding to nitrobenzene, at approximately the 40 ppb level. There also appeared to be about 5 ppb of 2,6-DNT present. Although no distinct peaks were observed for isophorone or 2,4-DNT, large peaks in their vicinity limited their detection to about 20 ppb. Based on the analysis of the unspiked extract, the sample was spiked with 100 ppb of nitrobenzene, 2,4-DNT, and isophorone and 50 ppb of 2,6-DNT. Results from the spiked samples before and after storage for seven days are shown in Table 17 and chromatograms for spiked and unspiked samples are shown in Figures 18 and 19. Recoveries were rather low and somewhat variable, especially for the DNTs. The results were compounded somewhat by the fact that the preserved and unpreserved (spiked) samples were taken from different one gallon containers, which were later found to have different levels of background interferences. A large peak eluting near isophorone prevented its determination in the spiked samples. However, by standard addition to the extract it was determined that the MDL for isophorone would be about 400 ppb. Similarly a large peak, perhaps a decomposition product of one of the background components, interfered with the determination product of one of the background components, interfered with the determination of 2,4-DNT in the preserved samples.

The clean up procedure did not improve the problem with background interferences as is illustrated by comparing Figure 19 to Figure 20. It was noted that for some sample aliquots, a yellow band was eluted from the column with methylene chloride, the first fraction to be discarded. For those aliquots, no recovery of the DNTs was observed, whereas for sample aliquots in which the yellow color was not eluted, higher recoveries of the DNTs were observed. This observation may be the result of overloading the column, although this is not certain. This result does serve to illustrate the need for each analyst to verify the elution profiles for the components of interest under his laboratory conditions, since variations can occur both within and between various laboratories.

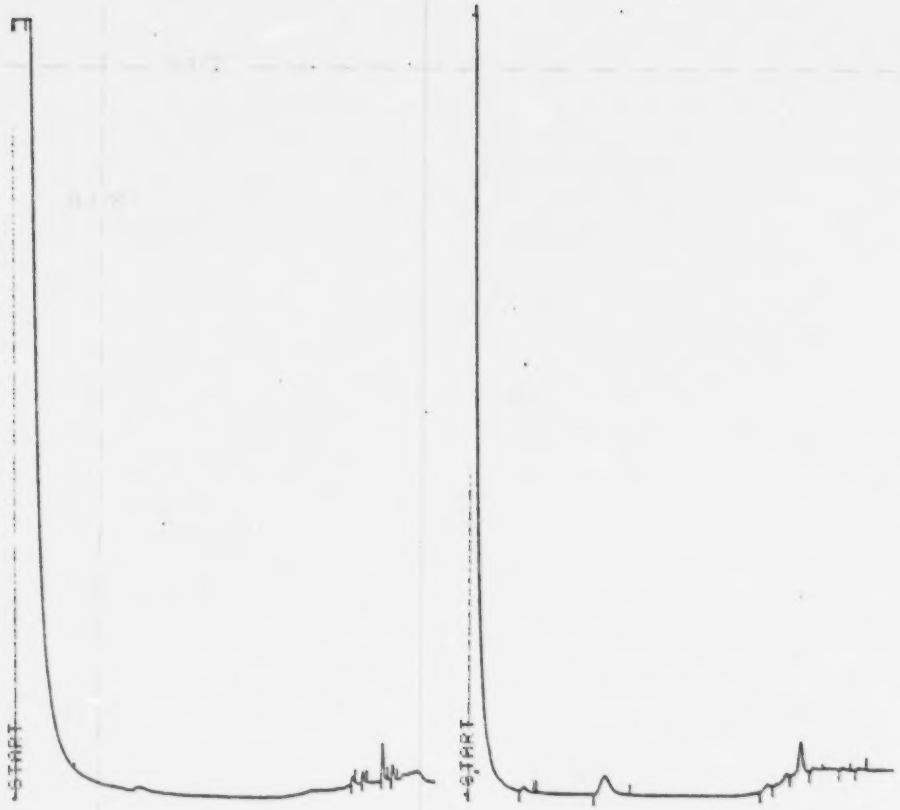


Figure 16. Chromatograms for Sample 3, unspiked, using FID at 85°C (a) and ECD at 145°C (b).

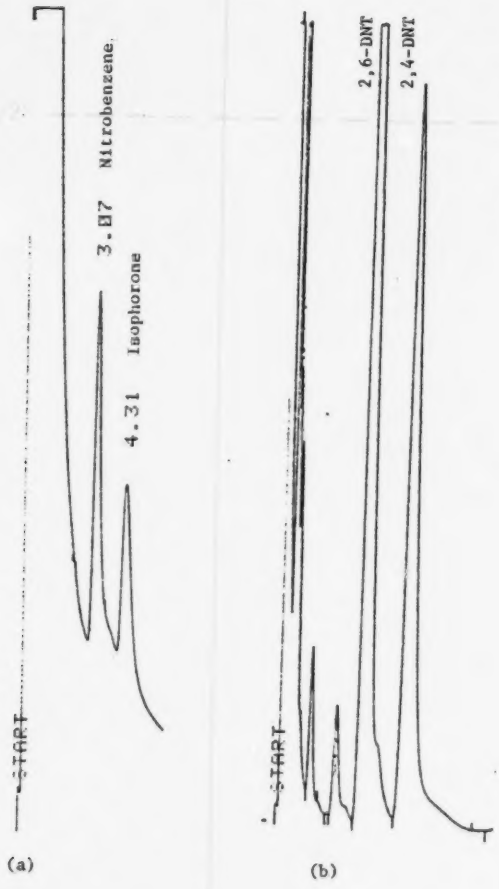


Figure 17. Chromatograms for Sample 5, spiked with 90 ppb of nitrobenzene, 50 ppb of isophorone, and 5 ppb of 2,4- and 2,6-DNT, using FID at 85°C (a), and ECD at 145°C (b).

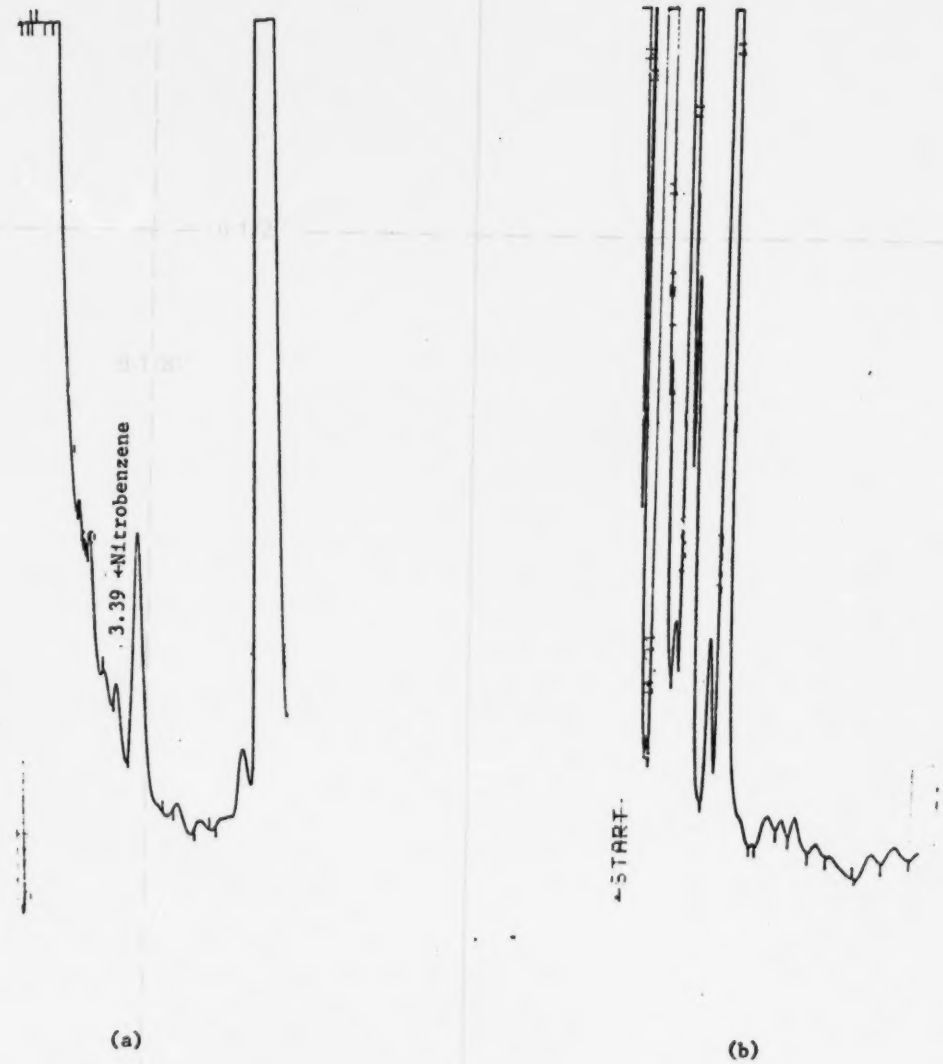


Figure 18. Chromatograms for Sample 6 unspiked using FID at 85°C (a) and ECD at 145°C (b).

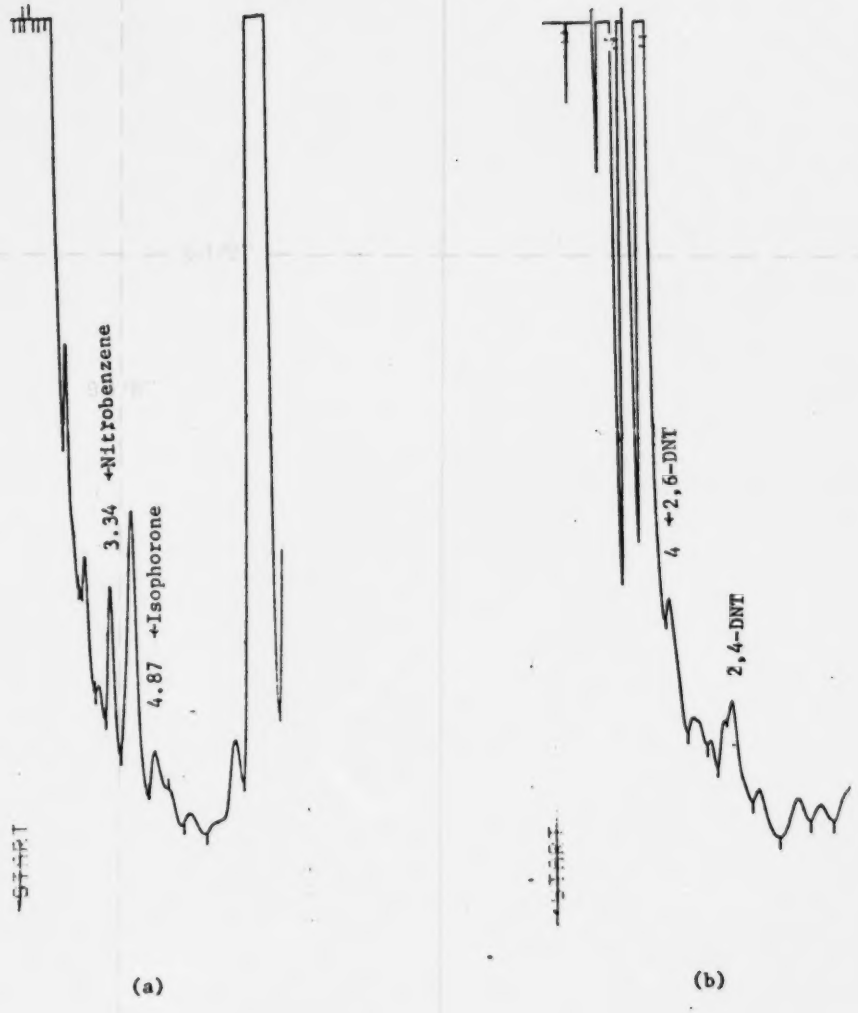


Figure 19. Chromatograms for Sample 6 spiked with 100 ppb of nitrobenzene, isophorone, and 2,6-DNT and 50 ppb of 2,4-DNT using FID at 85°C (a) and ECD at 145°C (b).

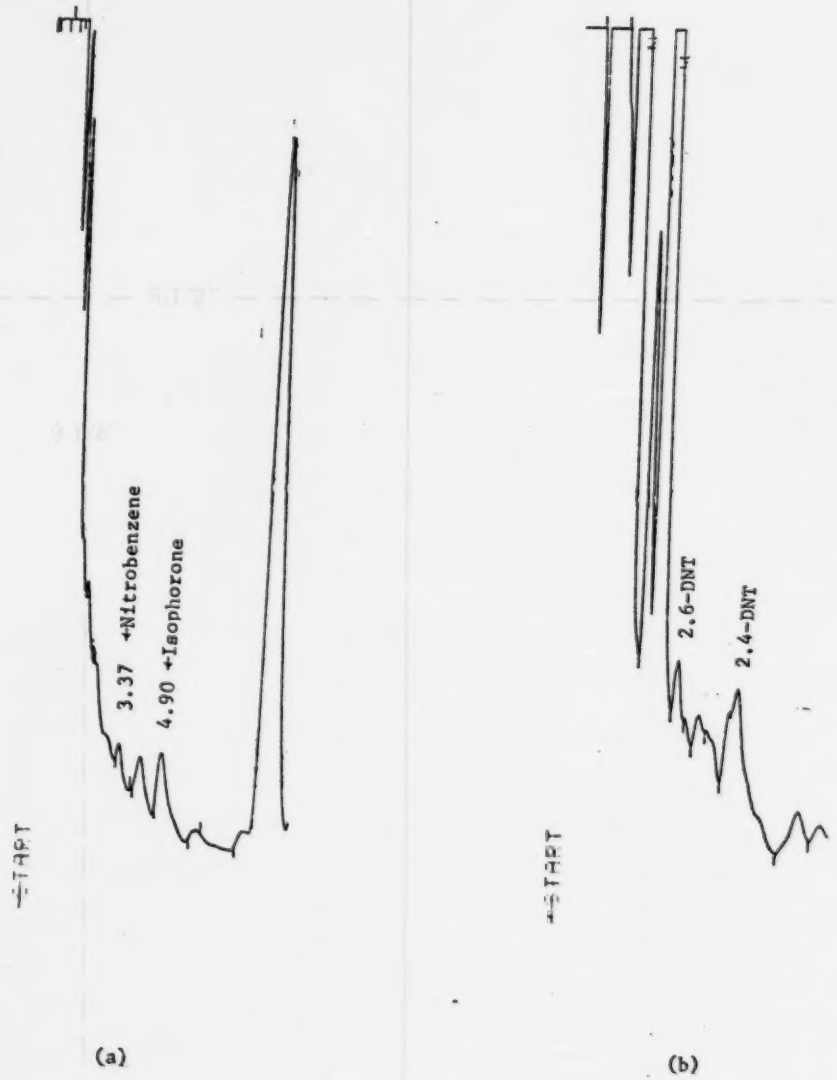


Figure 20. Chromatograms for Sample 6 spiked with 100 ppb of nitrobenzene, isophorone, and 2,6-DNT and 50 ppb of 2,4-DNT after florisil clean-up using FID at 85°C (a) and ECD at 145°C (b).

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SECTION 5

SUMMARY AND RECOMMENDATIONS

The results of this work are summarized below:

- The components of interest are readily chromatographed on OV-17 using the ECD for 2,4- and 2,6-DNT and the FID isophorone and nitrobenzene.
- The compounds are highly stable for at least 90 days when stored in either acetone or methanol.
- The compounds are readily extracted from waters at pH 7 with methylene chloride.
- The compounds are relatively stable for at least seven days when stored at pH 7 and 4°C, with or without chlorine present.
- Although the final method appeared to work very well for several aqueous samples, some problems were encountered with certain samples. Primarily, these problems were associated with high background levels of interfering components which limited the sensitivity of the measurement. Low recoveries were noted for several samples, the cause(s) of which are not known at this time.
- Special attention must be paid to the florisil clean up step in order to assure that the compounds of interest are eluting in the proper fraction.

On the basis of these results, it must be concluded that while the final method, given in the Appendix, appears to be satisfactory for many sample types, it must be validated for any particular application where it is to be used. In particular, each analyst must ensure that the extraction efficiency and clean up elution profiles for the sample are functioning properly. It is anticipated that for certain sample types, more efficient columns, for example, glass capillary columns, and/or more specific detectors, such as mass spectrometry, must be employed to achieve satisfactory quantitation for these specific compounds in the pesence of much larger amounts of interferences.

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APPENDIX

NITROAROMATICS AND ISOPHORONE

METHOD 609

1. Scope and Application

1.1 This method covers the determination of certain nitroaromatics and isophorone. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
2,4-Dinitrotoluene	34611	121-14-2
2,6-Dinitrotoluene	34626	606-20-2
Isophorone	34408	78-59-1
Nitrobenzene	34447	98-95-3

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL, defined in Section 14.1)[1] for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in Method 606, 608, 611 and 612. Thus, a single sample may be extracted to measure all of the parameters included in the scope of each of these methods. When cleanup is required the concentration levels must be high enough to permit selection of aliquots of the extract, as necessary, to apply appropriate cleanup procedures. The analyst is allowed the latitude, under Gas Chromatography (Section 12), to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

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1.5 Any modifications of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is extracted with methylene chloride using separatory funnel techniques. The extract is dried and exchanged to hexane during concentration to 1.0 mL by evaporation. Isophorone and nitrobenzene are measured by flame ionization gas chromatography (FIDGC). The dinitrotoluenes are measured by electron capture GC (ECGC)[2].

2.2 The method provides a Florisil chromatographic cleanup procedure to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned[3]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminates PCB interferences. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

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3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional clean-up approaches to achieve the MDL listed in Table 1.

4. Safety

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[4-6] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, 1-liter or 1-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

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5.2.2 Drying column - Chromatographic column, 400 mm long x 19 mm ID with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish - Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials - Amber glass, 10 to 15-mL capacity, with Teflon lined screw-cap.

5.2.8 Chromatographic column - 100 mm long x 10 mm ID, with Teflon stopcock.

5.3 Boiling chips - approximately 10/40 mesh. Heat to 400°C for 30 min. or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 1.2 m (4 ft.) long x 2 mm or 4 mm ID, Pyrex glass, packed with Gas-Chrom Q (80/100 mesh) coated with 1.95% QF-1/1.5% OV-17. This column was used to develop the method performance statements given in Section 14. Guidelines for the use of alternate column packings are provided in Section 12.1.

5.6.2 Column 2 - 3.0 m (10 ft.) x 2 or 4 mm ID, Pyrex glass, packed with Gas-Chrom Q (80/100 mesh) coated with 3% OV-101.

5.6.3 Detector - Flame ionization. This detector has proven effective in the analysis of wastewaters for isophorone and nitrobenzene, and was used to develop the method performance

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statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1.

5.6.4 Detector - Electron capture. This detector has proven effective in the analysis of wastewaters for the dinitrotoluenes, and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Sodium hydroxide solution (10 N) - (ACS) Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.3 Sulfuric acid solution (1+1) - (ACS) Slowly, add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.4 Acetone, hexane, methanol, methylene chloride, - Pesticide quality or equivalent.

6.5 Sodium sulfate - (ACS) Granular, anhydrous. Purify by heating at 400°C for 4 hrs. in a shallow tray.

6.6 Florisil - PR grade (60/100 mesh), purchase activated at 1250°F and store in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 200°C in glass containers loosely covered with foil.

6.7 Stock standard solutions (1.00 µg/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in pesticide quality hexane, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality

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control check standards that can be used to determine the accuracy of calibration standards, will be available from the U. S. Environmental Protection Agency, Environmental Monitoring and Systems Laboratory, in Cincinnati, Ohio.

6.7.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish gas chromatographic operating conditions to produce resolution of the parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane. One of the external standards should be at a concentration near, but above, the method detection limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences.

Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane. One of the standards should be at a concentration near, but above, the method detection limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1} \quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard, (µg/L).
C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant (< 10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check

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on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. After January 1, 1982, ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated

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in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

8.2.5 After January 1, 1982, the values for R and s must meet rigid method performance criteria provided by the U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

$$\text{Upper Control Limit (UCL)} = R + 3s$$
$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3.

The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance. After January 1, 1982, the control limits above must be replaced by method performance criteria provided by the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly[7].

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that

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all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices [8] should be followed, except that the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction[2].

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 5 to 9 with diluted sodium hydroxide or sulfuric acid.

10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool,

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centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the Kuderna-Danish if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Sections 10.7 and 10.8 describe a procedure for exchanging the methylene chloride solvent to hexane while concentrating the extract volume to 1.0 mL. When it is not necessary to achieve the MDL in Table 2, the solvent exchange may be made by the addition of 50 mL of hexane and concentration to 10 mL as described in Method 606, Section 10.7.

10.7 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation.

10.8 Add 1 to 2 mL of hexane to the concentrator tube, and a clean boiling chip. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of hexane to the top. Place this micro K-D apparatus on a water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column

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and rinse its lower joint into the concentrator tube with a small volume of hexane. Adjust the final volume to 1.0 mL and stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. Unless the sample is known to require cleanup, proceed with gas chromatographic analysis.

10.9 Determine the original sample volume by refilling the sample bottle to the mark with water and measuring the volume in a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 Prepare a slurry of 10g of activated Florisil in methylene chloride in hexane (1 + 9)(V/V). Use it to pack a 10-mm ID chromatography column, gently tapping the column to settle the Florisil. Add 1 cm of anhydrous sodium sulfate to the top of the Florisil.

11.2.1 Just prior to exposure of the sodium sulfate layer to the air transfer the 1 mL of sample extract onto the column using an additional 2 mL of hexane to complete the transfer.

11.2.2 Just prior to exposure of the sodium sulfate layer to the air, add 30 mL of methylene chloride in hexane (1 + 9)(V/V) and continue the elution of the column. Elution of the column should be at a rate of about 2 mL per minute. Discard the eluate from this fraction.

11.2.3 Next elute the column with 30 mL of acetone/methylene chloride (1 + 9)(V/V) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Concentrate the collected fraction by the K-D technique prescribed in Sections 10.6, 10.7, and 10.8 including the solvent exchange to 1 mL of hexane. This fraction should contain the nitroaromatics and isophorone.

11.2.4 Analyze by gas chromatography.

12. Gas Chromatography

12.1 Isophorone and nitrobenzene are analyzed by injection of a portion of the extract into an FIDGC. The dinitrotoluenes are analyzed by a separate injection into an ECGC. Table 1 summarizes some recommended gas chromatographic column materials and operating conditions for the instruments. This Table includes retention times and MDL obtained under these conditions. Other packed columns,

chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, the analyst must not add the internal standard to sample extracts until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 2 to 5 µL of the sample extract using the solvent-flush technique [9]. Smaller (1.0 µL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2.} \quad \text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:
A = Amount of material injected, in nanograms.
V_i = Volume of extract injected (µL).
V_t = Volume of total extract (µL).
V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used,

calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

$$\text{Eq. 3.} \quad \text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:
A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract (µg).
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 Method detection limits - The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero[1]. The MDL concentrations listed in Table 1 were obtained using reagent water[10]. Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of recovery from reagent water and has been demonstrated to be applicable over the concentration range from 7 x MDL to 1000 x MDL[10].

14.3 In a single laboratory (Battelle, Columbus Laboratories), using spiked wastewater samples, the average recoveries presented in Table 2 were obtained[2]. Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the percent recovery is also included in Table 2.

14.4 The Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

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TABLE 1

CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

Parameter	Retention Time (min.) Column 1	Column 2	Method Detection Limit (µg/L) EC	FID
Nitrobenzene	3.31	4.31	13.7	3.6
2,6-Dinitrotoluene	3.52	4.75	0.01	-
Isophorone	4.49	5.72	15.7	5.7
2,4-Dinitrotoluene	5.35	6.54	0.02	-

Column 1 conditions - Gas-Chrom Q (80/100 mesh) coated with 1.95% QF-1/1.5% OV-17 packed in a pyrex glass column 1.2 m (4 ft) long x 2 mm or 4 mm ID. Nitrogen carrier gas at a flow rate of 44 mL/min was used when determining isophorone and nitrobenzene by FID. The Column temperature was isothermal at 85°C. Methane (10%)/Argon (90%) carrier gas at flow rate of 44 mL/min was used when determining the dinitrotoluenes by ECGC. The column temperature was isothermal at 145°C.

Column 2 conditions - Gas-Chrom Q (80/100 mesh) coated with 3% OV-101 packed in a pyrex glass column 3.0 m (10 ft) long x 2 mm or 4 mm ID. Nitrogen carrier gas at a flow rate of 44 mL/min was used when determining isophorone and nitrobenzene by FID. The column temperature was isothermal at 100°C. Methane (10%)/Argon (90%) carrier gas flow rate of 44 mL/min was used when determining the dinitroltoluenes by ECGE. The column temperature was isothermal, 150°C.

A 2 mm ID column was used with the FIDGC and a 4 mm ID column was used with the ECGC.

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BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
2,4-Dinitrotoluene	63	3.1	5-100	21	4
2,6-Dinitrotoluene	66	3.2	5-50	24	4
Isophorone	73	4.6	50-60	21	4
Nitrobenzene	71	5.9	90-100	24	4